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# THE JOURNAL OF AGRICULTURAL SCIENCE

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## THE ORGANIC MATTER IN HEAVY ALKALINE SOILS.

By A. F. JOSEPH AND B. W. WHITEFELD.

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### I. INTRODUCTION.

THE Sudan soils—loess and alluvial—which have formed the subject of communications to this *Journal* during recent years are almost always low in organic matter, so low indeed as to suggest the question as to whether its presence is of any importance for fertility. This is one of the problems which the investigations now recorded were designed to answer and whilst the function and importance of these small amounts of humus are still uncertain, a great deal of information has been collected which it is desirable to record.

The total amount of organic matter varies, of course, very greatly with the nature of the soil and the climatic conditions. The only soil met with containing a quantity which would be considered normal in a temperate climate, was from the bank of the Upper White Nile (Bahr-el-Gebel) under marshy conditions: this sample contained 4.7 per cent. organic carbon. The usual amount however is well below 1 per cent.

The determinations of organic carbon were made by wet or dry combustion method on samples which had been freed from carbonates by treatment with hydrochloric acid. The wet combustion method originally used was that of Waynick<sup>(1)</sup> but the later determinations have been made almost exclusively by the dry method, using a silica combustion tube heated electrically.

Typical results for the percentage of carbon obtained are as follows; the figures relating to one or more 3- or 4-foot holes:

Table I.

Shambat (near Khartoum)	0.29	Dongola	0.44
Gezira Research Farm	0.23	Malakal	0.37
Hag Abdulla (Southern Gezira)	0.42	Sobat River	0.77
Makwar (near Sennar Dam)	0.47	Abu Zabad, Kordofan	0.09

All the soils were heavy alkaline clays, except the last, which was a light red sandy soil (*goz*).

The conventional method of assuming "loss on ignition" to represent

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organic matter is quite useless for heavy soils which often contain much more combined water than organic matter, and as determinations of total carbon do not lend themselves to field studies on any scale, it became essential to adopt a simple method for humus estimation, not only to enable large numbers of samples to be dealt with, but also because it is generally considered that the alkali-soluble organic matter is of more importance than the total.

A very brief examination of the methods in use for humus determination convinced us that the only possible one for our purposes was one based on colorimetry owing to the large number of approximate determinations required, and we have consequently modified the method for clay soils described by Beam<sup>(2)</sup> by the introduction of various simplifications which make the method very similar to that of Eden<sup>(3)</sup>. Before however deciding on this method, it was considered necessary to examine a few heavy soils of different types in order to obtain information as to (a) the ultimate composition of the humus from different soils and (b) the stability of standard humus solutions.

### 2. SOILS FROM WHICH HUMUS PREPARATIONS WERE MADE.

The three soils used as a source of humus were:

(a) No. 12161, the recently deposited "silt" taken from the Blue Nile at Khartoum.

(b) No. 12805, a first-foot sample from the Gezira near Wad Medani, about 100 miles south of Khartoum and a mile or so west of the Blue Nile.

(c) No. 11789, a sample taken at a depth of 4 ft. from near the river bank of the Bahr-el-Gebel about 800 miles south of Khartoum.

The composition of these soils was as follows:

Table II.

	Stones and gravel	Coarse sand	Fine sand	Silt	Clay	Organic carbon
	%	%	%	%	%	%
Blue Nile	0.0	0.8	57.6	25.5	16.1	1.28
Wad Medani	2.1	6.0	11.6	19.3	60.8	0.30
Bahr-el-Gebel	0.0	0.0	21.1	24.4	54.4	4.77

### 3. PREPARATION OF HUMUS.

About 10 kg. of soil were treated with dilute hydrochloric acid for about 24 hours in order to decompose carbonates and humates, and the acid liquor removed by several washings by decantation. The residue was then extracted repeatedly with 1 per cent. caustic soda solution

until the solution was no longer dark brown. The humus was then precipitated by the addition of acid, washed, redissolved in caustic soda solution, and the solution filtered through a collodion filter to remove the small quantity of clay always carried down in the preliminary operations. The humus was then reprecipitated by acid, filtered and washed, and finally treated with dilute hydrofluoric acid to remove siliceous matter. After further washing, it was dried *in vacuo* at the room temperature over calcium chloride. The preliminary operations take up a good deal of time owing to the small amount of organic matter present in these soils and the bulky nature of the suspensions, but we were anxious to obtain preparations as free as possible from mineral impurities. We have obtained in this way three preparations with an ash content of less than 1 per cent., an amount very much smaller than that recorded, for example, by Snyder (4) for four different samples, the ash content of which varied from 4 to 12 per cent. Cameron and Breazeale (5) also referred to the "considerable amount of mineral matter" ordinarily present in humus preparations.

It may be that the small quantity of "mineral matter" which our samples contain forms part of the real humus, as we were unable to reduce the ash content by further treatment with hydrochloric and hydrofluoric acids.

The results of the analyses are as follows, the carbon, hydrogen and nitrogen being calculated on the ash-free material.

Table III.

	Ash	Carbon	Hydrogen	Nitrogen	Oxygen (by diff.)
Blue Nile	0.36	57.37	4.74	3.59	34.30
Gezira	0.83	57.60	3.43	2.29	35.68
Bahr-el-Gebel	0.35	57.44	5.34	3.01	34.21

The proportion of carbon is remarkably constant considering the rather widely different character of the origin of the samples.

#### 4. PERMANENCE OF THE COLOUR OF HUMUS SOLUTIONS.

For many purposes, a standard humus solution is more convenient for comparative colorimetry than standard glasses, and experiments have therefore been carried out to ascertain the conditions which might lead to alteration in the colour of a standard solution.

The standard solutions are made by dissolving 0.1 gm. of humus in about 10 c.c. of *N*/10 sodium hydroxide and diluting to 1000 c.c.: they are then matched against standard glasses (No. 1 blue, No. 1 red, and No. 2 yellow) used with the Dubosq colorimeter.

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Solutions kept in the dark at the temperature of the laboratory (20°–35°) do not undergo much change, a 0.012 per cent. Nile humus solution giving the following readings against standard glasses:

Table IV.

	April 1923 (fresh made)	Sept. 1923 5 months	April 1924 12 months
Colorimeter reading	2.12	2.23	2.46
Relative colour	100	95	86

Exposure to diffused light exerts no greater effect, but bright sunlight causes a rapid falling off in colour intensity as is illustrated by the following results for the same standard solution kept for eight weeks (a) in the dark, (b) in diffused light, (c) in bright sunlight. From the above it is seen that in the dark the change in two months is small: the solutions exposed to light were tested against that kept in the dark, the colour intensity of which is put equal to 100. The table includes results for the same solution kept in an oven at 70°, the solution being contained in a small flask closed by a mercury trap.

Table V.

Days ...	0	11	20	29	43	56
Diffused light	100	100	100	100	100	100
Bright light	100	73	67	64	59	56
70° C. ...	100	100	90	90	83	83

The last results suggest that the air originally in the flask rather than the temperature, is responsible for the colour change. The experiment was consequently tried of exposing two portions of the same standard solution to bright sunlight in sealed tubes, one of which was half full of air, whilst from the other the air had been removed by boiling under reduced pressure, the tube being sealed during the boiling operation. The two tubes were exposed on the roof from February 28th to April 15th (6 weeks), at the end of which period the vacuum one showed practically no change, whilst the colour in that containing air had faded to one-fourth its original value. These results are sufficient to warrant the use of moderately fresh standard humus solutions for colorimetric purposes provided that reasonable precautions are taken against access of light and of air.

#### 5. THE COLORIMETRIC ESTIMATION OF HUMUS IN SOIL SAMPLES.

It should of course be understood that what is being estimated is that portion of the organic matter which is extracted from an acid-treated soil by means of cold dilute alkali, whose colour is in nature and intensity similar to that of the purified humus referred to above.

The method as adopted at present is as follows:

One gram of the soil is treated in a centrifuge tube with dilute hydrochloric acid to decompose calcium and magnesium carbonates and humates, and is then washed in the centrifuge until free from acid. A measured quantity (50 c.c.) of 4 per cent. sodium hydroxide is then added, and the soil shaken up and allowed to stand for 24 hours. The tube is centrifuged until the liquid is clear, and its colour then compared in the colorimeter with a standard solution of humus prepared from a similar soil.

It is in this important respect that our method differs from that of Eden (*loc. cit.*), who used as a standard a solution of Merck's *acidum huminicum*. At present we are not satisfied that preparations of humus separated from soils of different origin possess the same colour, and this point is being further investigated. Meanwhile, the use of humus solution prepared from soil samples similar to those under investigation, appears to be the least open to objection.

Six colorimeter readings are taken, for which the average difference from the mean is generally about 1 per cent.

Duplicate determinations on carefully mixed samples generally agree within 1-2 per cent., whilst in the case of replicate determinations made by withdrawing six portions from the soil bag, the average difference for the mean was found to be 4 per cent. in one case and  $2\frac{1}{2}$  per cent. in another.

## 6. HUMIFICATION.

The conditions leading to humification in this country have not yet been studied in detail: considerable variations exist from field to field, although groups of fields of the same fertility closely resemble one another. Humification appears to proceed with great difficulty under fallow conditions, as is shown by the following experiment carried out at Shambat (near Khartoum) in which a good lubia crop was ploughed in and subsequently disc harrowed, the usual irrigation being given at intervals of 15 days. The field was sampled before ploughing in the green crop, and at regular intervals afterwards, five samples being taken on each occasion and made into one composite for determinations of the total organic carbon and of humus. The results were as follows, the humus carbon being calculated from the humus, using the factor 0.576 (see Table III above).

It thus appears that, except for a rather sudden change in the early part of the experiment, very little humification took place under these

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conditions. The humus factor (ratio of humus carbon to total carbon) varied considerably, being 36 per cent. in July and only 27 per cent. in December.

Table VI.

	Before ploughing March 19	After harrowing						
		June 1	June 28	July 30	Sept. 1	Oct. 4	Nov. 4	Dec. 5
Humus ... ..	0.197	0.169	0.199	0.207	0.218	0.218	0.225	0.182
Humus carbon ...	0.113	0.097	0.114	0.119	0.126	0.126	0.130	0.105
Total organic carbon	0.188	0.476	0.383	0.332	0.343	0.358	0.360	0.395

As far as the Gezira is concerned, the humus factor for grouped soils is remarkably close to 40 per cent.: this is shown for the results of determinations of humus and total organic carbon for three composites each representing many samples:

Table VII.

Source	No. of samples com- pounded	Humus %	Humus carbon %	Total organic carbon	Ratio.
Good plots in Block 12	40	0.230	0.132	0.327	0.403
Bad plots in Block 12	40	0.199	0.115	0.289	0.396
Plots in Blocks 4 and 7	16	0.271	0.156	0.393	0.396

These results were obtained for samples in comparatively small areas: the results were less uniform in the case of composite samples taken to 4 ft. from 42 good and bad plots scattered over the Gezira. The following table includes some figures for individual holes with usually high and low humus content.

Table VIII.

Good plot high in humus	4	0.420	0.242	0.433	0.558
Good plot low in humus	4	0.190	0.109	0.419	0.260
Bad plot high in humus	4	0.394	0.227	0.406	0.487
Bad plot low in humus	4	0.081	0.047	0.256	0.183
Composite good plots	60	0.306	0.176	0.387	0.455
Composite bad plots	60	0.231	0.133	0.369	0.361

The humus factor varies considerably for the individual plots in which the total organic carbon varies much less than the humus. The mean result for the composite samples is nearly 40 per cent., as in the case of the first-foot samples.

## 7. THE VERTICAL DISTRIBUTION OF ORGANIC MATTER.

In the Gezira and other localities where the heavy soil is deep and approximately uniform, the soil profile shows two changes; the wide cracks characteristic of the *badob* become insignificant or disappear

altogether at about the fifth foot, at which depth the colour usually becomes distinctly lighter. The other change is that at about the third or fourth foot where there is a zone a foot or more thick where the colour is grey rather than brown. The diminution of colour in the lower layers is reflected in a lower content of organic matter, as is illustrated by the results for the following three deep holes:

Table IX.

Total organic carbon.

	Average for	
	1st 4 ft	2nd 4 ft
Gezira Research Farm (Wad Medani)	0.240	0.140
Makwar (Sennar Dam) ... ..	0.440	0.205
Abdin (above Makwar) ... ..	0.396	0.127

In the Gezira, the humus content passes through a well-defined maximum at the fourth foot: this is illustrated from the results averaged for 7 six-foot holes at Remeitab (Lat. 14.10, Long. 33.25):

Table X.

Depth	1st	2nd	3rd	4th	5th	6th
Humus	0.174	0.186	0.208	0.217	0.190	0.106

In this series of samples, the grey soils were somewhat irregularly distributed: of the 42 samples, there were 11 grey samples in the upper three feet, and 14 in the lower three, and the grey colour was certainly associated with the higher humus content.

Table XI.

	1st to 3rd ft.	4th to 6th ft.
Grey samples	0.194	0.208
Brown samples	0.183	0.097

The degree of humification varies with the depth rather irregularly, but so many properties are varying at the same time that it is not surprising that the results are not simple.

The following table gives the results for composite samples made by mixing the samples taken at the same depth from 6 six-foot holes dug two miles apart along a line in the middle of the Gezira 14° 27' N. The carbon and humus are given and also the figures for total nitrogen which are of interest in connection with the next section; the analytical particulars of the samples are also given.

The mean humus ratio is 42 per cent. which is quite near the above figures for other composite samples. It may be noticed that if the



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samples had been compounded into 3 two-foot sets (*i.e.* first and second, third and fourth, fifth and sixth), the ratios would have been 43, 41 and 41.5 per cent. respectively.

Table XII.

Depth	1	2	3	4	5	6
Clay % ...	56.0	55.9	59.4	60.6	59.5	51.3
Salts % ...	0.093	0.102	0.327	0.413	0.392	0.398
pH ...	9.10	9.17	9.10	8.89	8.97	8.87
Total nitrogen	0.0255	0.025	0.027	0.032	0.030	0.022
Humus ...	0.244	0.198	0.250	0.270	0.167	0.099
Humus carbon	0.141	0.114	0.144	0.156	0.096	0.057
Total carbon	0.248	0.352	0.341	0.389	0.348	0.101
Humus ratio %	56.8	29.6	42.2	40.2	27.6	55.4

### 8. VARIATION OF HUMUS CONTENT OVER AN APPROXIMATELY UNIFORM AREA.

During the last 2 years, a soil survey has been completed of the irrigated Gezira area of 300,000 acres, samples being taken every 2 min. of latitude and longitude. In this way 120 holes were sampled, usually to a depth of 6 ft., and the humus determined in all first-foot samples. The results for clay, water-soluble salts and humus vary with the position, the clay and humus being highest and the salts lowest in the central region.

Table XIII.

Latitude	Average % of		
	Clay	Salts	Humus
14° 43' to 14° 29'	51.2	0.234	0.222
14° 27' to 14° 15'	58.6	0.188	0.281
14° 13' to 14° 3'	53.5	0.233	0.221

There is at present not sufficient known as to any small climatological differences which may exist to frame an hypothesis as to the cause of these variations, but the figures for salts and humus suggest that there may be a connection between these constituents depending on the relation, well-established in this area, between salt content and crop yield (8). The less favourable environment for plant growth in the more salty places would obviously lead to a smaller accumulation of organic matter, and the connection is well shown by classifying the samples according to their clay content and then comparing the salts and humus. The results are shown below, those for clay and salts being the means for the first 4 ft. and those for humus being for first-foot samples only.

The relation between the salts and humus conforms roughly to the line  $\text{Humus} = 0.513 - 1.25 \times \text{salts}$ , the last column in the table showing the extent of the agreement.

Table XIV.

No. of holes	Range of clay content	Mean % of clay	Mean % of salts	Mean % of humus	Humus calculated
15	49 to 52	50.5	0.249	0.201	0.202
31	52 to 55	53.6	0.238	0.227	0.216
14	55 to 57	56.2	0.204	0.232	0.258
15	57 to 60	58.2	0.194	0.264	0.271
13	60 to 63	61.6	0.183	0.282	0.284
14	63 to 69	64.8	0.170	0.312	0.301

The connection between humus and salts implies one between humus and yield, and this is well shown by such figures as are given in Tables VII and VIII. It is possible by comparing the fertility of plots of nearly the same salt and clay content, to show that when these other variables are kept constant, yield and humus are associated. Such data are not easy to obtain, but the following series of 8 plots (in Table XV) under cotton support this view: the yields are given in lb. of lint per acre.

Table XV.

No.	Clay %	Salts %	Humus %	Yield	No.	Clay %	Salts %	Humus %	Yield
18268	51.0	0.219	0.335	426	18292	62.6	0.190	0.260	64
18275	54.4	0.190	0.280	348	18293	56.5	0.204	0.222	267
18277	55.2	0.225	0.323	327	18304	55.7	0.213	0.216	201
18295	57.0	0.186	0.266	271	18308	57.0	0.204	0.187	201
Mean	54.4	0.205	0.301	343		57.9	0.203	0.221	184

## 9. THE NITROGEN CONTENT OF THE SOIL.

Studies in the amount and variation of nitric nitrogen in the soil have already been reported by Martin and Massey<sup>(6)</sup> from field experiments at Shambat near Khartoum. The results obtained there apply in general to the Gezira: whilst the proportion of nitric nitrogen varies enormously in quite a small area and even in successive portions taken from the same sample bag, the variation in total nitrogen content is small. Systematic examination for ammoniacal and nitrous nitrogen have not yet been made but may safely be assumed to form only a very small part of the nitrogen. The following results were obtained for a number of samples taken from four-foot holes, each hole being sampled at each foot and the four samples mixed. The humus nitrogen is calculated from the analytical results in Table III showing that Gezira humus contains 2.29 per cent. nitrogen.

If the humus and nitric nitrogen be subtracted from the total, the amount left (189) may be regarded as the albuminoid nitrogen, and this from the average composition of 18 vegetable proteins given by Osborne<sup>(7)</sup> (52.11 per cent. carbon, 17.68 per cent. nitrogen) corresponds to 1070

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parts per million, containing 557 parts of protein carbon. Now the average for the total carbon content of these soils is about 0·3 per cent. or 3000 parts per million, so that the protein carbon constitutes about one-sixth of the total.

On the whole, the total nitrogen content of these soils varies very little. Thus, 42 composite samples (from which the 15 in Table XVI are selected) were divided into two groups according to the fertility of the fields which they represented and the following results obtained:

Table XVI.

Ref. No.	Parts per million			
	Total nitrogen	Humus*	Humus nitrogen	Nitric nitrogen
18272	350	3390	78	3
75	310	2800	64	7
86	440	3940	91	226
94	280	2240	52	113
96	290	1900	44	81
68	240	2580	59	34
69	290	3350	77	28
71	250	3070	71	4
76	290	2400	55	34
87	300	3940	91	6
90	260	2600	60	14
92	250	2600	60	19
99	330	2610	60	3
18302	300	2000	46	6
06	300	2160	50	8
Mean	299	2772	64	46

\* It should be remembered that "humus" includes only that part of the organic matter soluble in cold alkali. The nitrogen contained in any other organic matter (*e.g.* that soluble in *hot* alkali) would be included in the albuminoid nitrogen.

Table XVII.

Parts per million		Mean for 25 good plots	Mean for 17 bad plots
Total nitrogen	...	304	295
Humus	...	3065	2310
Humus nitrogen	...	70	53
Total organic carbon		3870	3690
Carbon nitrogen ratio		12·7	12·5

The amounts of total nitrogen are, of course, exceedingly small, being only about one quarter of those given by Russell<sup>(9)</sup> for barren wastes: the ratio of carbon to nitrogen is however the same as that given for English soils<sup>(10)</sup>. The nitrogen content is a good deal less than the average recorded for 7 first-foot samples of African soils by Shantz<sup>(11)</sup>, for which the average is 940, and is also much less than that recorded by Hall<sup>(12)</sup> for 54 South African soils, which averaged 1120 parts per million. It is nearer to that recorded by Bal<sup>(13)</sup>, who found 250 to 1000 parts per million for a number of heavy Indian soils.

## SUMMARY.

1. Sudan soils are very low in organic matter, the total organic carbon being usually less than 1 per cent. In the Gezira, the humus carbon is about 40 per cent. of that of the total.

2. Humus preparations purified as far as possible could not be obtained ash-free. Specimens of humus from widely different sources contain nearly the same proportion of carbon.

3. Humus solutions (in very dilute alkali) keep fairly well in the dark. They also keep in bright sunlight if air is excluded. The use of standard solutions for colorimetric purposes is justified if not kept too long.

4. Field studies show that the humus content of good soil is greater than that of poor, and that there is a marked inverse connection between salt and humus content.

5. The above conclusion does not apply to the depth distribution of these constituents. In the Gezira, the maximum humus content is found at the fourth foot and the maximum salt content is found at about the same depth.

6. The total nitrogen content of the soils studied is low, usually about 0.03 per cent. About one-fifth of this is humus nitrogen, and the carbon-nitrogen ratio is about twelve to one.

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# THE MOISTURE EQUIVALENT OF HEAVY SOILS. II.

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THE investigations previously recorded in this *Journal*<sup>(1)</sup> dealt mainly with the effect of electrolytes on moisture equivalent, alkalinity and the "colloidal" nature of the clay. The work which forms the subject of this paper was designed to investigate the extent to which the moisture equivalent of a soil was an additive property dependent on the nature of the fractions, subject of course to the modifications caused by the soil electrolytes and possibly humus.

Attention was drawn to one case where two soils from the same area closely resembling one another with respect to mechanical composition and electrolyte content had moisture equivalents of 37.5 and 49.0—a very large difference. Since then, many other cases of this kind have been met with of which the following are examples:

Table I.

Sample No.	Clay %	Silt %	Salts %	Moisture equivalent
7896	14.1	5.0	0.015	8.6
8924	10.6	4.2	0.090	17.1
13107	76.1	10.1	0.003	35.7
7556	63.9	12.8	0.045	56.3
6640	57.2	10.8	0.047	35.5
11499	36.7	7.3	0.033	43.5

In the case of each pair, the lower sample gives the highest moisture equivalent, whereas the mechanical composition suggests that the upper one should have done so.

Amongst the factors which have in the past been considered to be closely associated with moisture equivalent, the humus and colloidal content of the soil are the most important. The former may be ruled out here, as all the above soils are low in humus: further, recent investigations have shown that no scientific precision can be attached to the term "colloidal content" as practically the whole of the clay fraction of an ordinary soil can be obtained in "colloid" form<sup>(2)</sup>. On the other

hand, the properties of clays (*i.e.* the inorganic soil colloids) differ very greatly, and have been shown to be connected to a very considerable extent with their chemical composition, and in particular with the ratio of silica to alumina(3). For clays separated and purified by similar methods, this ratio is closely associated with "clay" properties which enter so greatly into their water relationships. In some cases the moisture equivalent follows closely the composition, being highest with clays highest in silica: in other cases, the differences are more strikingly exhibited when the effect of heat or of alkalis on moisture equivalent is studied(3), or better, the imbibitional water content<sup>1</sup>. This constant shows a much better relationship with composition (and with clay-like properties) than moisture equivalent. Thus some bodies which are relatively deficient in plasticity (*e.g.* kaolin) have quite high moisture equivalents: a high imbibitional water content has, however, not yet been observed with such substances.

The technique of determining the imbibitional water on a small quantity of clay is not very satisfactory, and does not always give good duplicates. It is, however, often not possible to provide 30–60 gm. of highly purified clay for the determinations, and a method had to be worked out for the use of much smaller quantities. This consists of substituting for the ordinary soil boxes, blocks of wood of the same size and shape having a central hole which takes a small cylindrical copper cup with wire gauze bottom, in which 3–4 gm. of material gives a layer of the necessary thickness. The blocks are weighted with lead, so that the whole when assembled weighs about the same as the ordinary soil box carrying 30 gm. of wet soil. The agreement obtained between the two methods is shown in the table below:

Table II.

Material used	Moisture equivalent	
	30 gm.	4 gm.
Soil 6640	37.2	37.8 and 34.2 = 36.0
Repeat	38.0	37.8 and 37.2 = 37.5
Clay 8379	60.0	59.5
Clay 9228	76.8	74.0
Clay 7900	37.8	37.4

The following table gives the imbibitional moisture capacity of pure clay fractions from widely different sources arranged in the order of their silica-alumina ratio in four groups, from which it will be seen that

<sup>1</sup> Cf. Fisher (4). The imbibitional water is the volume retained by 100 c.c. of soil less the volume of xylene retained by the same soil after the standard treatment with the moisture equivalent apparatus.

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although there are many exceptions, on the average, a high imbibitional moisture capacity is associated with a high silica-alumina ratio.

Table III.

Ref. No.	Origin	Silica- alumina ratio	Imbibitional moisture	Mean
10118	China clay, Cornwall	2.11	5.0	$\text{SiO}_2/\text{Al}_2\text{O}_3$ = 2.51 Imb. aq. = 53.8
15577	Orangeburg loam, Miss.	2.20	79.0	
13107	Mongalla, Sudan	2.41	53.8	
10120	Ball clay, Cornwall	2.66	39.5	
10468	Sub-soil clay, N. Wales	2.84	101.6	
7900	Red sandy soil, Kordofan	2.85	43.8	
8320	Kassala alluvium	3.77	79.2	$\text{SiO}_2/\text{Al}_2\text{O}_3$ = 3.92 Imb. aq. = 105.9
6640	Sudan Gezira ( <i>badob</i> )	3.90	100.8	
9223	Tokar alluvium	3.93	110.5	
6960	Kordofan Khor soil	3.95	117.7	
15421	White Nile alluvium	3.96	121.0	
14637	Sudan Gezira ( <i>badob</i> )	4.16	143.0	
15576	Wabash silt loam, Nebraska	4.29	134.0	$\text{SiO}_2/\text{Al}_2\text{O}_3$ = 4.34 Imb. aq. = 114.3
9650	Potting clay, Luxor	4.28	69.6	
7897	Brown sandy soil, Kordofan	4.41	96.8	
16177	Blue Nile alluvium	4.53	128.0	
10743	Bentonite, B. Columbia	6.96	261.0	$\text{SiO}_2/\text{Al}_2\text{O}_3$ = 7.35 Imb. aq. = 311.5
10744	Bentonite, Alberta	7.67	362.0	

## PROPERTIES OF COLLOID CLAY SUB-FRACTIONS.

The fact that the inorganic colloid content of a soil is practically identical with the clay content raises the question as to how far this fraction of a soil is homogeneous. The efforts made so far to separate one or two of our typical clays into fractions differing in chemical composition and physical properties have been only partly successful. The most complete experiment made consisted of passing a suspension of purified *badob* clay through the super-centrifuge, thus separating the clay into colloid and residue. The residue was shaken with water and again centrifuged when a further portion of colloid was obtained. As has been pointed out (2), practically the whole of the clay can thus be obtained in the colloid form.

This was separated from the liquid by passing it through the centrifuge at a much lower rate—1-2 litres per hour—by which treatment it was divided into three portions: the finest still passed the centrifuge and was separated from its very dilute suspension on a collodion filter. The clay left in the bowl was divided—following Bradfield (5)—into two portions, the denser or suspensoid, and the lighter, more translucent, emulsoid. These three were then subjected to full chemical analysis and

determination of imbibitional water capacity with the following results: the results of the chemical analysis are abbreviated:

Table IV.

	Original clay	Suspen- soid	Emulsoid	Collodion filtered
Silica ... ..	47.48	47.93	48.06	46.89
Aluminium ... ..	19.38	18.64	18.99	19.14
Ferric oxide ... ..	13.40	14.85	13.90	13.34
Lime + magnesia ... ..	4.94	3.83	4.28	3.99
Soda + potash ... ..	1.78	1.15	1.14	1.09
Volatile matter ... ..	10.55	12.55	13.61	15.09
Silica-alumina ratio ... ..	4.16	4.36	4.29	4.16
Moisture equivalent by weight...	68.7	78.3	85.7	96.5
Xylene equivalent by weight ...	11.7	10.9	9.6	10.5
Imbibitional moisture by volume	143.0	171.0	193.0	229.0

The alkalis which seemed to disappear, presumably pass in the form of silicates and aluminates through the collodion filter. The differences in imbibitional water content are out of all proportion to those in chemical composition, nor can they be due to differences which only exist in the suspensions, because the moisture equivalent determinations were made on the dried fractions. The important but exceedingly laborious experiment remains to be made, of attempting to convert the suspensoid and emulsoid forms into the finest form, using the method by which the original clay can be almost entirely converted into colloid. Meanwhile, it must be noted that important differences in moisture equivalent may be obtained with materials of approximately the same ultimate chemical composition.

#### INFLUENCE OF REPLACEABLE BASES ON MOISTURE EQUIVALENT.

Another factor which has been found to affect considerably the water-holding properties of purified clay is the relative calcium-sodium content. This was investigated by treating portions of clay with sodium and calcium chloride, enough of the respective hydroxides being added to make the mixtures alkaline. After standing some days, the suspensions were dialysed until the specific resistance rose to well over 10,000 ohms: the pure calcium and sodium clays were then examined by chemical analysis and for moisture equivalent. The results were as follows:

Table V.

	Original clay	Calcium clay	Sodium clay
Silica ... ..	47.48	46.53	47.92
Aluminium ... ..	19.38	18.93	17.82
Calcium oxide ... ..	0.77	2.30	0.59
Sodium oxide ... ..	1.19	0.43	1.55
Silica-alumina ratio ... ..	4.16	4.17	4.56
Moisture equivalent by weight...	68.7	63.0	125.0
Xylene equivalent by weight ...	11.7	11.1	11.4
Imbibitional water by volume...	143.0	131.0	292.0



The introduction of calcium and sodium reciprocally into the clay is well shown.

It is unfortunate that the treatment altered the silica-alumina ratio as well as the proportion of bases, but it is unlikely that this would be sufficient explanation of the very large rise in water-holding capacity. The results are, in fact, in agreement with other familiar properties of clay saturated with calcium and sodium respectively.

#### MOISTURE EQUIVALENT AS AN ADDITIVE PROPERTY.

The investigations of Middleton(6) on this subject led to the formulation of an equation connecting moisture equivalent with mechanical analysis as follows:

$$\text{Moisture Equivalent} = 0.426 \text{ clay} + 0.291 \text{ silt} + 0.063 \text{ sand.}$$

If this is to hold over a complete range of mechanical composition, it implies that all clays have a moisture equivalent of 42.6 and all silts of 29.1 and, as this is not the case, the relationship cannot be general; and in fact, it does not apply to the soils dealt with in this paper. Thus for the six soils in Table I, the values calculated from the above formula differ on the average by 10.7 from those observed and in only one of the six is there reasonable agreement. It is, however, true that the moisture equivalent of a mixture is an additive property in simple cases, and this was established by testing in the ordinary way a number of mixtures of pure clay and clean ignited sand. The results are given below, the last column giving the result calculated from the formula: Moisture Equivalent = 0.595 clay + 0.032 sand derived from the first and last lines:

Table VI.

Clay %	Sand %	Moisture equivalent	Moisture equivalent calculated	Difference
0	100.0	3.2	—	—
16.7	83.0	14.8	12.5	+2.3
33.3	66.7	24.8	21.9	+2.9
50.0	50.0	33.7	31.2	+2.5
66.0	40.0	36.7	37.0	-0.3
83.3	16.7	46.7	49.9	-3.2
100.0	0	59.5	—	—
			Mean	2.2

This is a very reasonable approximation to the simple linear relation involving no constants except the moisture equivalent of the two components.

Series of soils of the same kind may show a fair agreement of this kind: this is illustrated by the following results for seven samples from Tokar

(near the Red Sea). The clay and silt fractions separated and purified were found to have moisture equivalents of 59.1 and 49.5 respectively and the last column gives the calculated values for the soils without allowing anything for the sand fraction:

Table VII.

	Clay %	Silt %	Moisture equivalent	Moisture equivalent calculated	Difference
Pure clay	100.0	0	59.1	—	—
Pure silt	0	100.0	49.5	—	—
Soil No. 10314	14.3	11.4	16.0	14.0	2.0
9220	27.4	22.2	24.1	27.3	3.2
9219	28.0	28.1	29.8	30.5	0.7
10327	49.7	19.4	37.0	39.0	2.0
10319	21.2	44.5	39.3	34.5	4.8
10323	51.0	30.5	43.5	45.2	1.7
9218	54.0	36.2	48.0	49.8	1.8

The average difference 2.3 is practically the same as in the case of the sand-clay mixtures.

There are obvious anomalies in these results: in several cases the calculated value is higher than the observed one in spite of no allowance having been made for any water-holding capacity of the sand fractions.

During a more comprehensive study of Gezira soils, series of samples were found in which those taken from the upper layers showed a relationship quite different from that observed with those taken lower down. In order to investigate this point further, the clay fraction from a number of samples taken at a depth of 10 ft. was separated and purified and its composition and properties compared with that from first-foot samples, from which it was found to differ considerably. The following figures show this:

Table VIII.

	SiO <sub>2</sub> /Al <sub>2</sub> O <sub>3</sub>	Moisture equivalent	Imbibitional water
Clay from 1st foot	3.90	57.5	101
Clay from 10th foot	4.91	78.6	163

It therefore seemed possible that better agreement with the empirical equation would be obtained if allowance were made for the higher moisture equivalent of the clay present in the samples taken at a low depth, and the next two tables show that this is the case.

Table IX below gives the results for six samples taken at a depth of 3 or 4 ft. (those from the first and second feet often showed water-logging), the calculated values being obtained from the formula

$$\text{Moisture Equivalent} = 0.575 \text{ clay} + 0.275 \text{ silt} + 3.$$

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The first two coefficients are the moisture equivalents of first-foot clay and silt, and the last an arbitrary figure added on account of the sand.

Table IX. *Third- and fourth-foot samples.*

Sample No.	Clay %	Silt %	Moisture equivalent	Moisture equivalent calculated
8843	54.1	15.4	36.3	38.3
8867	57.2	15.1	37.3	40.0
8891	57.5	16.0	42.1	40.5
8915	53.0	12.7	38.5	37.0
8868	58.2	13.2	42.5	40.0
8892	60.5	14.5	41.2	41.5

The mean difference is 1.6. The next table contains the results for six samples from the seventh or eighth foot with the calculated values according to the above formula, and also when the coefficient for clay is taken as 0.786, 78.6 being the moisture equivalent of the clay separated from the tenth-foot samples<sup>1</sup>.

Table X. *Seventh- and eighth-foot samples<sup>1</sup>.*

Sample No.	Clay %	Silt %	Moisture equivalent	Calculated (i)	Calculated (ii)
8847	36.2	14.7	36.6	27.8	35.2
8871	30.9	26.4	34.1	28.1	34.4
8895	38.5	26.6	39.6	32.4	40.3
8919	42.6	11.3	36.5	30.6	39.5
8943	40.0	8.8	34.3	28.4	36.6
8872	27.0	33.3	33.7	27.7	33.3

The average differences are 6.5 using the first formula, and 1.3 for the second.

It seems, therefore, that for a complete study of a soil, it will be necessary to make a detailed examination of the properties and composition of the clay fraction separated from samples taken at different depths.

### THE SILT FRACTION IN RELATION TO MOISTURE EQUIVALENT.

Much of this paper deals with the fact that the nature as well as the amount of clay is an important factor in determining the properties of a soil. A more detailed examination of certain soil types has shown further that the silt fractions may differ amongst themselves as much as the clays, and that this must be taken into consideration when attempting to formulate additive relationships. The results so far obtained indicate that the two classes of soil studied here—aeolian and alluvial—

<sup>1</sup> The tenth-foot samples contain too little clay to furnish the desired information as to which method of calculation gives the most satisfactory results.

differ much more in respect of their silt than their clay fractions, both in chemical composition and moisture equivalent.

The following table for first-foot samples gives the results obtained so far: it should be borne in mind that the classification into wind-borne and water-borne soils is not necessarily the most logical for these soils, as the three alluvial ones are all transported from the Abyssinian mountains, and the three aeolian ones from the north-west desert. It may therefore be a question of original composition rather than method of transportation.

Table XI.

Type	Origin and reference No.	Silica-alumina ratio		Moisture equivalent	
		Clay	Silt	Clay	Silt
Alluvial	Kassala 8320	3.77	4.13	59.5	55.6
	Tokar 9223	3.93	4.47	55.6	49.5
	Blue Nile 16177	4.53	5.92	66.0	49.3
	Mean ... ..	4.08	4.84	60.4	51.5
Aeolian	Kordofan 7900	2.85	9.67	41.8	39.0
	Kordofan 6960	3.95	8.12	56.5	34.5
	Gezira 6640	3.90	7.13	57.5	27.5
	Mean ... ..	3.57	8.31	55.3	30.3

It is clear that the differences in composition and properties of the silts are much greater than in the case of the clays, and detailed study of the silt would appear to be essential in the case of soils containing a high proportion of this fraction.

My thanks are due to Mr O. W. Snow for making many of the chemical analyses required for these investigations.

#### SUMMARY.

1. The moisture equivalent of pure clay preparations varies with (a) the chemical composition, (b) the method of separation if centrifuged, (c) the replaceable bases.

2. The imbibitional water content also shows a close connection with the above variables.

3. Good additive relationships can only be obtained from series of soils of the same nature and in some cases, if taken at the same depth. This is, at any rate in part, due to differences existing in composition and properties between clay separated from soils and subsoils.

4. Pure silt fractions from different soils showed marked differences in their chemical composition and moisture equivalent.

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## THE LENGTH OF THE INTERVAL BETWEEN CALVINGS.

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### INTRODUCTION.

IT being generally accepted that a high yield per cow is one of the most potent factors that make for financial success in dairying, the question arises as to how frequently a cow should calve, so that her average weekly yield over a long period may be at a maximum. The general form of the cow's lactation curve, as is well known, shows that the yield rises slightly for a few weeks after parturition, and then falls fairly steadily throughout, except for more or less minor irregularities caused by seasonal influences, etc.; it is also common knowledge that too frequent calving, and too short periods of rest (which, in practice, are closely related), undermine the cow's constitution, and, in some cases, reduce her yield to a much lower plane. The farmer has, therefore, to steer a course between the Scylla of pushing the cow too far, and the Charybdis of allowing her to spend a large part of her milking life on the tail-end of a lactation curve.

It is recognised that, in practice, a cow is often served so that she may calve when her milk is wanted, and also that the exact date of service is not by any means entirely under human control; consequently no great exactitude is required, but the above question is often asked, and could the optimum interval between calvings be determined, the knowledge would serve a useful purpose as a general guide.

The prevailing view is that a cow should calve at approximately yearly intervals; the average interval for 1410 lactations recorded by the Penrith M.R.S.(2) was found to be 15 days over the year, and for 3918 Norfolk lactations(4),  $1\frac{1}{2}$  days under the year; the variation, however, was very wide.

Hammond(1) states that there is a great loss of milk where the cow does not calve again within the year, and Mackintosh(3) that it is customary to try and arrange for a cow to have a calf per annum; the latter considers it a debateable point whether or not it is advisable to allow longer periods to heavy milkers, because of the difficulty and risk of drying them off. Wing(6) says "practically all dairymen are agreed that

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milk is produced under the most favourable conditions, when the cow produces a calf at regular intervals each year, and this regardless of the value of the calf when born."

The term "service period" is used to denote the time from calving to the next fertile service, and it will be convenient to designate the interval from one calving to the next, the "calving interval"; it will readily be seen that  $\text{calving interval} = \text{service period} + \text{length of gestation}$ ; the latter has been taken as 280 days, and consequently a calving interval of one year corresponds to a service period of 85 days.

### THE DATA.

The writer has extracted the figures for all complete lactations collected by the Norfolk M.R.S., and has carried out a statistical analysis of them, with the object of measuring the effects of various factors on the yield of milk in a lactation. These data contain the records of from one to seven lactations of particular cows, and the first method of attack was to take the histories of cows of which several lactations were known, and to divide them into groups according to the average calving interval; the total actual yield in the known lactations of each cow was then divided by the number of weeks occupied by her lactations, and hence the average weekly yield over the total period found.

It was also possible to arrive, independently, at a theoretical curve showing the effect, by means of the standardising corrections that have been found necessary to allow for the three factors (excluding feeding and management), which most largely affect the cow's lactation yield—the length of the service period, the age of the cow, and the length of the period of rest, or dry period, preceding the lactation.

It is, of course, only possible to attack the problem with the "average cow" as the basis; this however is what is needed to establish any general principle, and it is hardly necessary to warn practitioners against the dangers of applying average results too slavishly to particular cases, as all progressive dairy farmers realise that, to obtain the best results, each cow must, to a certain extent, be treated individually.

### RECORDS FROM THE NORFOLK DATA.

The above-mentioned Norfolk data contained the following records:

- Series I.    1st and 2nd lactations of 101 cows.
- Series II.   1st, 2nd and 3rd lactations of 44 cows.
- Series III.  1st, 2nd, 3rd and 4th lactations of 36 cows.
- Series IV.   Three lactations between 3rd and 9th of 68 cows.

No cows appear in more than one of these series—*e.g.* the first two lactations of cows in Series III were not included in Series I—as it was thought that a better check would be obtained by having four entirely separate series. In the case of Series IV it will be noticed that a fairly wide age-range is covered; it includes the “maximum” lactation (the 6th) and three lactations on either side of it; in the few cases where there were more than three lactations of the cow available in this series, those were taken which were nearest to the 6th.

Each series was divided into four groups, according to the total number of weeks involved in the milking periods dealt with, and the

Table I. *Effect of Length of Calving Interval on Mean Weekly Yield.*

SERIES I. (Mean of 25 cows in Groups A, B and C, 26 cows in Group D.)						
Group	Mean service period in days	Mean time in weeks from 1st to 3rd calving	Mean total yield in two lactations (lb.)	Mean weekly yield (lb.)	Mean “mature standard” of cows included (lb.)	Corrected mean weekly yield (lb.)
A	38.6	91.0	10,805	119.2	7921 ± 86	122.4
B	58.4	96.7	12,470	127.4	8508 ± 93	121.7
C	76.0	101.7	12,410	122.0	7891 ± 86	125.7
D	134.6	118.5	14,523	122.6	8237 ± 90	121.1
SERIES II. (Each Group includes 11 cows.)						
Group	Mean service period in days	Mean time in weeks from 1st to 4th calving	Mean total yield in three lactations (lb.)	Mean weekly yield (lb.)	Mean “mature standard” of cows included (lb.)	Corrected mean weekly yield (lb.)
A	49.1	141.0	17,730	126.0	8262 ± 111	130.0
B	67.2	148.8	20,739	139.5	8801 ± 118	135.0
C	82.1	155.2	20,027	129.0	7990 ± 107	137.1
D	104.3	164.7	24,047	145.9	9072 ± 122	136.7
SERIES III. (Each Group includes 9 cows.)						
Group	Mean service period in days	Mean time in weeks from 1st to 5th calving	Mean total yield in four lactations (lb.)	Mean weekly yield (lb.)	Mean “mature standard” of cows included (lb.)	Corrected mean weekly yield (lb.)
A	53.0	190.3	25,663	134.9	8328 ± 107	124.6
B	70.7	200.4	27,442	136.9	8152 ± 105	129.6
C	81.8	206.7	25,659	124.1	7096 ± 92	134.4
D	106.8	221.0	27,742	125.0	7382 ± 95	130.7
SERIES IV. (Each Group includes 17 cows.)						
Group	Mean service period in days	Mean time in weeks occupied by three lactations	Mean total yield in three lactations (lb.)	Mean weekly yield (lb.)	Mean “mature standard” of cows included (lb.)	Corrected mean weekly yield (lb.)
A	54.8	143.5	22,970	160.0	8685 ± 94	154.6
B	76.2	152.6	24,232	158.8	8588 ± 93	155.3
C	87.4	157.4	26,386	167.6	8831 ± 95	159.1
D	127.0	174.4	24,398	140.3	7508 ± 81	155.3



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mean weekly yield over the whole period determined for each group. Thus in the four groups of Series I there were 25 cows (26 in the case of Group D), in those of Series II, 11 cows, in Series III, 9 cows, and in Series IV, 17 cows. The results are shown in Table I.

In the first column of the table is given for each group of each series the mean service period, and from this was obtained the total number of weeks occupied by the specified lactations (next column); *e.g.* in the case of Group A, Series I, mean s.p. = 38.6 days, and consequently the total time from 1st to 3rd calving was  $\frac{2 \times 38.6 + 560}{7} = 91.0$  weeks. Then follows the mean amount of milk produced per cow in the whole period, and, by division, the mean weekly yield.

It will at once be realised that the numbers available were insufficient to give an even distribution of "milking ability" over the various groups; fortunately however allowance could be made quite accurately for this as corrections had previously been obtained(2) for standardising a lactation yield; these corrections give an estimate from any lactation record of what that particular cow will yield in a normal lactation, when at her prime (*i.e.* the 6th lactation), the estimate so obtained being subject to probable error of 7.72 per cent. From each lactation included here the cow's "mature standard" was calculated, and the mean "mature standard" for each group is given in the table, together with the probable error of the estimate; for the latter we have, in the case of the four groups in Series III, for example,  $\frac{7.72}{\sqrt{4} \sqrt{9}} = 1.29$  per cent., there being four lactations of each cow and nine cows in each group.

From the smallness of the probable errors, it will be seen that the mean "mature standards," for the various groups, give very accurate comparisons of the milking abilities of the cows contained in them; in each series there are considerable differences between the groups—for instance in Series III, Groups A and B include much heavier milkers than Groups C and D. The mean weekly yields were therefore corrected by the mean "mature standards" of the corresponding groups, the method being to express each of the latter as  $\pm$  a percentage of the mean of the four in the series, and apply these percentages to the corresponding mean weekly yield; *e.g.* in Series I the mean of 7921, 8508, 7891, and 8237 is 8139, and the first of these is 2.7 per cent. below this mean—accordingly 2.7 per cent. was added to 119.2, giving the figure 122.4.

This correction, in every case, gives a fairly smoothly running set of figures, and the agreement between the series is very close; it will be

seen that the mean weekly yield rises from Group A to Group C and falls again in Group D, there being only one exception to this, namely, a slight fall from Group A to Group B in Series I.

Taking the arithmetic mean of the corrected mean weekly yields for the four series, we obtain the following result:

Group	Mean service period (days)	Mean calving interval (weeks)	Mean weekly yield (lb.)
A	48.9	47.0	132.9
B	68.1	49.7	135.4
C	81.8	51.7	139.1
D	118.2	56.9	135.9

The average weekly yield appears therefore to be markedly influenced by the frequency of calving; too short calving intervals depress the average considerably, as also do too long ones. The optimum interval is a little difficult to determine from these figures, but, apparently, it is not less than twelve months.

Taking the uncorrected mean weekly yields in the same way, and also the arithmetic mean "mature standard" yields, we have:

Group	Mean weekly yield (lb.)	Mean "mature standard" yield of Group (lb.)
A	135.0	8299
B	140.6	8512
C	135.7	7952
D	133.4	8050

These results do not tally with those given above for corrected mean weekly yields—here the suggestion is that the optimum lies in the neighbourhood of a service period of 70 days (*i.e.* a calving interval of 50 weeks); two serious objections might, however, be raised against these figures. Firstly, the mean "mature standard" yields show that Groups A and B contain higher milking cows than Groups C and D; clearly direct comparison between these groups is not justified. Secondly, no sort of consistence appears in Table I, in the uncorrected mean weekly yields for the four different series—*e.g.* the maximum occurs in Group B for Series I, Group D for Series II, Group B for Series III and Group C for Series IV—and the variations within the series are quite erratic; on the other hand with corrected mean weekly yields, all four series have the same tale to tell. The writer, therefore, decided to reject the uncorrected figures and accept the corresponding results with corrected mean weekly yields, as giving, probably, a closer approximation to the truth; as is

pointed out below (p. 32) the conclusion is, to a certain extent, dependent on the acceptance of the standardising corrections, as making accurate allowance for the various factors; the probable error of the "mature standard" estimate was, however, obtained directly from the estimates arrived at from successive lactations of particular cows, and not in any theoretical manner; the smallness of these probable errors definitely shows that the cows in Groups A and B were superior to those in Groups C and D.

#### THEORETICAL CURVE.

As has been said, a table of standardising corrections has been worked out, by means of which allowance may be made for variations in age, and in the lengths of service and dry periods, and a "mature standard" yield calculated from one lactation yield; from this table it is possible to work in the reverse direction, and estimate what an average cow with a "mature standard" yield of, say 8000 lb. (roughly, the mean of the Norfolk cows), would yield under different sets of conditions. The other measurable factor influencing the yield—the month of calving—has been omitted as its effect is small compared to the others, and clearly it would only complicate a question in which great accuracy is not needed.

From this correction table has been calculated what our average cow would produce in each of her first six lactations, with different lengths of service periods—*i.e.* these yields have been calculated separately, with six successive service periods of 10 days, of 20 days, of 40 days... up to 200 days.

The date of service determines the date of next calving, and there is a close correlation between the length of the service period and the length of lactation; the correlation coefficient between these last two variables has been found to be  $+0.795 + 0.008$  in the case of first calf heifers, and  $+0.758 + 0.005$  in the case of older cows; the regression of length of lactation on length of service period is quite linear and is given by the following equations:

$$\text{1st calvers } y = 35.7 + 0.105x \quad \text{.....(1)}$$

$$\text{Others } y = 33.3 + 0.109x \quad \text{.....(2)}$$

where  $y$  = length of lactation in weeks and

$x$  = length of service period in days.

From these equations it was possible to find the length of lactation associated with the various service periods, and hence the lengths of the dry periods were obtained.

The following will serve as an example of the method: consider the 3rd lactation yield of the average cow ("mature standard" yield = 8000 lb.) which has a service period of 100 days in each of her first six lactations. Her calving interval for all lactations will be  $100 + 280 = 380$  days and the length of lactation (except 1st lactation), as given by equation (2) above, will be  $33.3 + (0.109 \times 100)$  weeks = 309.4 days; hence the dry period before the 3rd (and subsequent) lactation will be  $380 - 309.4 = 70.6$  days. In order to obtain the "mature standard" yield of this cow the following corrections would have to be applied to her 3rd lactation yield:

For service period of 100 days  $\rightarrow - 3.5$  per cent.

For age (3rd lactation)  $\rightarrow + 9.3$  per cent.

For dry period of 70.6 days  $\rightarrow - 5.9$  per cent.

For practical purposes it is permissible to add these percentages together, but for greater accuracy they should be put in the form  $(1 + x)$  and multiplied; in this way, the total correction here would be

$$0.965 \times 1.093 \times 0.941 = 0.992.$$

Hence 3rd lactation yield of above cow  $\times 0.992 = 8000$  lb.

3rd lactation yield = 8065 lb.

The first six columns of Table II show the lactation yields arrived at in this way taking successively all six service periods at 10, 20, 40...200

Table II. *First Six Lactation Yields of "Average cow"*  
with Different Lengths of Service Period.  
(Cow with "Mature Standard" Yield = 8000 lb.)

Length of service period in each lactation (days)	Calculated lactation yields (lb.)						From 1st to 7th calving		
	1st	2nd	3rd	4th	5th	6th	Total yield of milk (lb.)	Time in weeks	Average weekly yield (lb.)
10	4778	4882	5586	5891	6066	6107	33,310	248.6	134.0
20	4969	5168	5895	6211	6395	6442	35,080	257.1	136.4
40	5358	5814	6510	6861	7067	7118	38,727	274.3	141.2
60	5702	6367	7080	7462	7685	7737	42,033	291.4	144.2
80	6033	6902	7598	8008	8247	8307	45,095	308.6	146.1
100	6334	7380	8065	8493	8753	8810	47,835	325.7	146.9
120	6628	7813	8602	8958	9227	9291	50,419	342.9	147.0
140	6867	8230	8879	9368	9638	9709	52,691	360.0	146.4
160	7105	8611	9227	9732	10,012	10,088	54,775	377.1	145.3
180	7326	8969	9558	10,075	10,376	10,444	56,748	394.3	143.9
200	7526	9291	9852	10,390	10,695	10,767	58,521	411.4	142.2

days; the next column gives the total of these six lactation yields, and the following one the time in weeks from the 1st to 7th calving—e.g. for

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service periods of 10 days this is  $\frac{(6 \times 10) + (6 \times 280)}{7} = 248.6$  weeks; finally, by division, was obtained the average weekly yield over the whole period, and these figures in the last column are represented in Fig. 1.

Table II demonstrates very forcibly the large effect which environment (in its widest sense) may have on the lactation yield; the standardising corrections have been calculated from the means of large numbers of

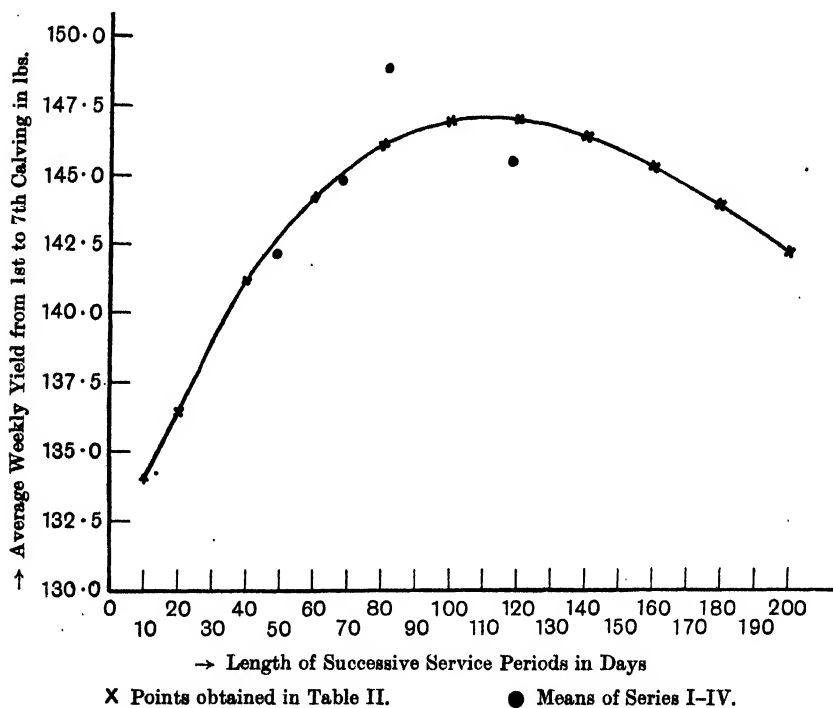


Fig. 1. Effect of Length of Service Periods on Average Weekly Yield.

records, and individual cases would show much greater variation, but even with the "average cow" the lactation yield will vary from 4778 lb. to 10,767 lb., within the limits taken for these three variables alone. This fact emphasises the necessity of some method of standardising a lactation yield for purposes of comparison.

As the corrections were obtained originally from smooth curves, it is not surprising to find that the points shown in Fig. 1 run quite smoothly. A curve has been drawn through them and it will be seen that successive

short service periods will lead to a greatly reduced average weekly yield over a long period, and successive long service periods will also lead to a low average. The optimum service period appears to be somewhere between 100 and 120 days, and the curve is approximately symmetrical about a service period of 110 days, except that the fall is slightly accentuated for service periods of less than 50 days. The mean weekly yield for service periods of 110 days is 147.0 lb. as opposed to 134.0 lb. for service periods of 10 days—a difference of nearly 10 per cent. in favour of the former.

It is interesting to compare the results obtained above for Series I-IV with the theoretical curve of Fig. 1—this comparison is made in Table III.

Table III. *Means of Series I-IV compared to Theoretical Curve.*

Group	Arithmetic mean service period (days)	Arithmetic mean weekly yield (lb.)	Corresponding weekly yield given by curve (lb.)	Mean of Series I-IV corrected to level of curve (lb.)
A	48.9	132.9	142.7	142.1
B	68.1	135.4	145.1	144.8
C	81.8	139.1	146.2	148.8
D	118.2	135.9	147.0	145.5
Mean		135.8	145.25	145.3

In this table the mean service periods and corrected mean weekly yields for the Series I-IV are repeated from p. 25; the next column gives the corresponding figures shown by the curve—*e.g.* the curve cuts the line for service period = 48.9 days at a weekly yield of 142.7 lb.; the means of the two corresponding sets of figures are 135.8 and 145.25 and consequently the figures from Series I-IV have, in the last column, been raised to the level of the curve by multiplication by  $\frac{145.25}{135.8}$ ; the figures obtained are represented in Fig. 1 by solid circles.

The agreement is surprisingly close—the points for Groups C and D, though departing from the curve give a mean for the two groups of 147.15 at a service period of 100 days—the curve gives the figure 146.9.

Despite this, however, it might be argued that the results for Series I-IV show an earlier maximum and an earlier falling off, Group D being below Group C. It is probable that this arises as a result of the departure of the relationship from linearity; Groups A, B and C embrace restricted ranges of mean service period, but Group D extends a long way—one cow in Series I, for instance, having a mean service period of 356 days. The mean is, of course, obtained in a linear manner, but the curve, as it

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progresses to the very long service periods, becomes steeper and steeper; cows in Group D with very long calving intervals, therefore, cause a lowering of the mean weekly yield disproportionate to the rise they cause in the mean service period of the group.

From the run of the four points, however, in Fig. 1, the optimum suggested is certainly not earlier than a service period of 81.8 days (Group C) and probably somewhere between 90 and 100 days; this compares well with the maximum of the curve at approximately 110 days. Without going into any unnecessary and unjustifiable detail, it is claimed that these results show that the mean weekly yield is at a maximum when the calving interval is slightly over the year—perhaps it would be more prudent to draw the more modest conclusion that cows should not calve at intervals of less than a year. The age variation probably depends directly on the age of the cow, rather than on the number of lactations she has had, and consequently the objection might be raised that the same corrections would not be applicable all through Table II; if this is so, however, it would merely emphasise the point made and tend to move the optimum further towards the long service periods, for these cows would have their later lactations, involved here, when they were nearer to their prime. The practical man usually arrives ultimately, by trial and error, at something like the optimum, and it has already been seen that the average calving interval is within a day and a half of a year in Norfolk and about a fortnight over the year in the Penrith district; both of these approximate well to the optimum found above, though there seems some danger of Norfolk farmers erring on the side of serving their cows too soon—this is the more apparent when it is pointed out that the distribution of length of service period is positively skew and one half of the Norfolk cows are served within 70 days of calving.

The problem is essentially an economic one, and consequently the results shown in Fig. 1 have been reduced to shillings and pence. Venn and Carslaw<sup>(5)</sup> found that the cost of keeping a cow for a year was £33. 9s. 9d; assuming an average annual yield of 600 gallons and that the food for a gallon of milk costs 3d., the annual cost of the production ration would be £7. 10s., leaving £25. 19s. 9d. as the total "cow-keep" cost (to include maintenance)—or almost exactly 10s. a week. In Table IV the cost of production of a gallon of milk, according to the calving interval, has been determined on the basis that it costs 10s. a week for "cow-keep" and 3d. more for the production ration for each gallon of milk (taken as 10 lb.). What the farmer wants is the greatest profit per

cow, and this profit has been calculated by valuing the milk at 1s. 4d. per gallon.

Table IV. *Cost per Gallon and Profit per week with different Lengths of Service Period in first six Lactations.*

Service period in days	Time from 1st-7th calving in weeks	Total yield in gallons (= 10 lb.)	Cost in shillings			Cost per gallon in pence	Value of milk at 1s. 4d. a gallon (shillings)	Profit per week (shillings)
			"Cow-keep" at 10s. a week	Production ration at 3d. per gallon	Total cost			
10	248.6	3331	2486	833	3319	11.06	4441	4.51
20	257.1	3508	2571	877	3448	11.79	4677	4.78
40	274.3	3873	2743	968	3711	11.50	5164	5.30
60	291.4	4203	2914	1051	3965	11.32	5604	5.62
80	308.6	4510	3086	1127	4213	11.22	6013	5.83
100	325.7	4784	3257	1196	4453	11.17	6379	5.91
120	342.9	5042	3429	1260	4689	11.16	6723	5.93
140	360.0	5269	3600	1317	4917	11.20	7025	5.86
160	377.1	5478	3771	1369	5140	11.26	7304	5.74
180	394.3	5675	3943	1419	5362	11.33	7567	5.59
200	411.4	5852	4114	1463	5577	11.43	7803	5.41

The weekly profit per cow, on this basis, rises from 4s. 6d. with service periods of 10 days to 5s. 11d. for service periods of 120 days, and falls to 5s. 5d. for service periods of 200 days. The same conclusion must be drawn if the prices are varied simultaneously or separately between 5s. and 15s. a week for "cow-keep," 2½d. and 3½d. as the cost of the production ration per gallon, and 1s. 2d. and 1s. 6d. as the value of the milk—namely, that the economic optimum coincides with the optimum weekly yield.

It has only been attempted to work with the "average cow," in order to establish the broad principle; it would appear probable that the optimum would vary slightly from cow to cow, according to individual persistence of yield—that it might be advisable to have slightly longer calving intervals with cows giving the flat type of lactation curve, and to serve a little earlier with cows which yield highly at first but fall off rapidly and dry off early. Reference should also be made to Mr Burkitt's statement<sup>(1)</sup> that cows should be served as soon as their yield drops below 4 gallons a day; clearly no hard and fast rule could be laid down which would be generally applicable, the individual optimum interval varying, both with the level, and with the persistency, of production of the cow. The whole skill in the application of average results to practice, lies in the ability to modify the application correctly to particular cases.

The tentative conclusion reached in this paper is that, with the



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"average cow," the calving interval should be not less than a year, and should, if anything, be a week or two over the year.

The results are, of course, based on the standardising corrections, that have been found necessary to allow for age, etc.; the difficulty is to obtain sufficient data and the correct details to give the relationship direct. The corrections, however, probably approximate very closely to the truth for the "average cow," since two sets obtained from two entirely different batches of records, confirmed one another almost exactly; it is thought that the corrections used contain no errors large enough, materially to affect the conclusion reached in this paper.

### SUMMARY.

An attempt has been made to ascertain the best interval between calvings, so that the cow's average weekly yield over a long period may be at a maximum. It was only possible to consider the case of the "average cow," but it is claimed that the general principle has been established, that cows should calve at intervals of not less than a year, and not more than thirteen months; this optimum will probably be subject to a slight variation in particular cases. This is approximately the state of affairs in practice—though Norfolk farmers appear to err on the side of serving too early in the lactation, rather than too late.

It is with great pleasure that the writer acknowledges his indebtedness to Mr J. Hammond, M.A., whose criticism—always helpful and never merely destructive—is greatly appreciated.

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## THE PROTEINS OF GREEN FORAGE PLANTS.

### III. THE PROTEINS OF FORAGE PLANTS OF THE NATURAL ORDER CRUCIFERAE (GENUS *BRASSICA*). COMPARISON WITH COLZALIN, A GLOBULIN FROM RAPESEED.

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IN a previous paper (Davies, 1926) the author studied the differences in the compositions of protoplasmic proteins of plants within a Natural Order (Leguminosae). The Natural Order Cruciferae afforded a means of developing the study in the direction not only of ascertaining differences, if any, of protoplasmic protein of plants within a *genus*, but also of differences possible within a *species*. Thus proteins were studied from the following varieties of the cabbage species (*Brassica oleracea* L.): Cabbage (*B. oleracea* var. *capitata*), Marrow stem kale (*B. oleracea* var.) from the stems and leaves separately, Kohl rabi (*B. oleracea* var. *caulorapa*). Also, the proteins from the leaves and roots respectively of white turnips (*B. napo-brassica*) were isolated and studied. In order to compare the protoplasmic proteins with those of seed of a plant of the same species, a globulin from rapeseed (seed of *Brassica Napus* L.) was prepared and analysed.

The plants were grown on the University Farm on the same type of soil and samples were taken when the plants were fully grown, so that correspondence in stage of growth gave confidence in comparing the proteins from each species.

The proteins from the green leaves of the particular plants worked on, were extracted by the following methods: The leaves were minced finely and the juice pressed out immediately by means of a powerful hand press, filtered through a pad of cotton wool, and heated to 70° in a water bath, after adding a few drops of acetic acid and some sodium chloride solution, so as to coagulate the protein. The press-cake was minced into a finer state of division, stirred to a paste with water and again pressed, the liquid thus obtained being treated in the same way as the original juice, and the coagula united. The coagulum so obtained was washed several times with boiling water so as to leach out any soluble material occluded during coagulation and also to granulate the coagulum further. The washed precipitate was collected in the reservoirs

of Soxhlet extractors by filtration through filter paper cartridges and subjected to exhaustive extraction with warm alcohol for 30 hours, then with absolute alcohol for 3 hours, and then with dry ether. During these extractions, large amounts of colouring matter were extracted which when isolated from the solvents appeared as a waxy dark green solid, completely soluble in alcohol and ether to form very dark green solutions which turned to a dark brown on making alkaline with caustic soda.

After sucking off the excess ether from the extracted preparations, in each case they fell away into dusty light green powders, which were ground thoroughly in warm mortars and further extracted with dry ether for three hours. The powders thus obtained were analysed for moisture, ash, and nitrogen contents. The results obtained are tabulated below (Table I).

Table I. *Analysis of preparations from the leaves of some Brassica species.*

Source	Moisture %	Ash %	Nitrogen %	Nitrogen (on ash- and moisture-free basis) %
Coagulum from cabbage	2.72	3.01	13.04	13.82
Marrow-stem kale leaves	5.04	3.46	12.89	14.09
Turnip leaves	4.36	7.51	14.01	14.92

The residual pulp, after extraction of the above liquids in the case of cabbage and turnip leaves (fermentation had set in too far with marrow-stem kale leaves), was further extracted several times with alkaline alcohol (2 per cent. caustic soda in 60–70 per cent. alcohol) at 70°, pressing out the extract each time, filtering through a pad of cotton wool and acidifying the filtrate. A copious green precipitate was formed which was treated in a similar way to the above coagulum. (In the case of cabbage an intermediate cold alkaline alcoholic extract was obtained and isolated in the same way.) These preparations were dried by the same process as the coagula and analysed (Table II).

Table II. *Analysis of alkaline alcohol-extracted protein from the leaves of some Brassica species, after separation of the aqueous extract.*

Source	Moisture %	Ash %	Nitrogen %	Nitrogen (on ash- and moisture-free basis) %
Cabbage (cold extract)	7.97	2.62	9.72	10.30
Cabbage (hot extract)	5.40	3.15	12.99	14.21
Turnip leaves (hot)	—	—	13.19	—

During the extractions a balance of the nitrogen was recorded, the results being tabulated in Table IV.

The proteins from the fleshy parts of the above plants together with kohl-rabi bulbs were extracted in the following manner: The tissue was finely minced and the juice immediately pressed out, filtered, and the protein coagulated at 70°. The weight of juice yielded by these pressings amounted to 70-75 per cent. of the weight of material taken. Two further aqueous extractions of the press-cake were carried out and the extracts treated in the same way. The coagulum thus collected was washed several times with boiling water and extracted for long periods with warm rectified spirits in a battery of Soxhlet extractors, then with absolute alcohol and finally dry ether, the preparations being finally isolated as creamy, dusty powders. These were further extracted with dry ether after thorough powdering and mixing. On isolation in a dusty, friable condition they were kept over sulphuric acid in a desiccator for 48 hours owing to previous experience of the difficulty of driving away all the ether at room temperature. They were analysed in the usual way (Table III).

Table III. *Analysis of proteins obtained by aqueous extraction from the fleshy storage parts of some Brassica species.*

Source	Moisture %	Ash %	Nitrogen %	Nitrogen (on ash- and moisture-free basis %)
Kohl-rabi	6.90	5.01	12.33	14.00
Marrow-stem kale stems	7.20	5.08	10.39	12.22
Turnip	2.09	8.62	12.22	13.67

Further extraction of the press-cake by means of hot alkaline alcohol only extracted very small amounts of protein matter.

Table IV. *Distributions of nitrogen in some plants of the Brassica species (percentages of total nitrogen).*

Plant	Extracted nitrogen %	Protein nitrogen %	Amide nitrogen %	Other nitrogen %	Un- extracted nitrogen %
Cabbage	84	21	9.3	53.7	16
Kohl-rabi	90	10	8.6	71.4	10
Marrow-stem kale leaves	91	14	9.7	67.3	9
Marrow-stem kale stems	60	10	8.3	41.7	40
Turnip leaves	95	29	10.2	55.8	5
Turnip roots	69	13	8.8	47.2	31

A balance of the nitrogen present in the plant was made out during the extractions with a view of determining the distribution of the nitrogen.

The amide nitrogen of the juice free from coagulable matter was determined by Sacchse's method—by hydrolysis of an aliquot portion of liquid for 4 hours with 4 per cent. hydrochloric acid and then distilling off the ammonia after adding excess of magnesium oxide. The preceding table (Table IV) gives the distributions in the various plants as determined. The "Protein nitrogen" was calculated from the actual yield of protein obtained in each case.

*Properties of the preparations.*

The preparations obtained answered to all the protein tests. The test for tryptophane was responded to by all the samples, but with not such a marked colour in the case of the hot alcoholic alkaline preparations. The proteins, on ignition, gave the usual smell of burnt feathers and left behind a small amount of white fluffy ash which was found to be mostly calcium sulphate and phosphate, with traces of iron and chloride. The proteins did not dissolve in 0.2 per cent. caustic soda, so that this reagent could not be used for their further purification. They dissolved slowly in 10 per cent. soda in the cold, being gelatinised before being dispersed. Hot alkali at once dissolved them but gelatinous precipitates were formed on acidifying.

*The distribution of nitrogen in the preparations.*

The distributions of nitrogen in the samples were carried out in duplicate by the van Slyke method, the following being the figures obtained.

Table V. *Distributions of nitrogen in various protein preparations from some plants of the Brassica species.*

	Amide N %	Humic N %	Arginine N %	Histidine N %	Lysine N %	Cystine N %	Amino N of filtrate %	Non-amino N of filtrate %	Total N recovered %
Coagula from									
Cabbage	7.49	3.82	13.33	8.29	7.35	1.18	47.05	10.93	99.44
Kohl-rabi	6.57	5.43	14.45	8.37	7.23	1.26	46.91	9.82	100.04
Marrow-stem									
kale leaves	6.90	3.66	14.72	8.02	7.78	1.35	47.14	11.01	100.58
Marrow-stem									
kale stems	7.53	5.29	15.10	7.56	6.91	0.98	46.97	10.13	100.47
Turnip leaves	6.95	5.17	13.67	8.63	7.52	1.02	49.30	8.12	100.38
Turnip roots	6.77	5.04	15.17	7.23	8.86	1.01	46.81	8.53	99.42
Alkaline extracts									
from									
Cabbage	7.03	3.63	9.38	11.48	5.68	1.32	52.32	8.40	99.24
Turnip leaves	7.46	3.86	11.70	7.70	6.15	1.08	54.36	6.74	99.05

Examination of Table V shows that the coagulable protein in the juices of these plants have practically identical distributions of nitrogen, the nitrogen allotted to the diamino acids and to proline and oxyproline

being fairly high, hence it is evident that the amounts of these acids present in the proteins are appreciably large. Taking the average of all the analyses, the proteins are calculated to contain approximately the following percentages of these amino acids:

Table VI A.

Arginine	Histidine	Lysine	Cystine	Proline, etc.	NH <sub>3</sub>
%	%	%	%	%	%
7.2	4.7	6.3	1.6	13 (approx.)	1.4

The alkali-extractable proteins from cabbage and turnip leaves (after extraction of the water-soluble proteins) contain less of the diamino acids, and from the analyses do not resemble each other so closely in composition as the water-soluble samples, differing slightly in their arginine and histidine contents, although the sums of these two acids are about the same. The percentages of diamino acids present in these proteins are:

Table VI B.

	Arginine	Histidine	Lysine	Cystine	Ammonia
	%	%	%	%	%
Cabbage	4.7	6.8	4.7	1.8	1.4
Turnip leaves	5.8	4.5	5.1	1.5	1.4

It seems as if these proteins were conjugated to some grouping with the result that they could not be extracted with water, but the action of hot dilute alkali split off the prosthetic groups and so rendered the proteins soluble. On studying Tables VI A and VI B it is noticed that both classes of protein contain (a) identical percentages of amide nitrogen, and (b) in each protein the ratio arginine to lysine is nearly unity and, except for the similar cystine contents, no other inferences are justifiable.

*The protein from rapeseed. The globulin—colzalin.*

In order to compare a seed protein from a plant of the genus *Brassica*, the protein of rapeseed (*Brassica rapa*) was worked on. Wholesome rapeseed meal, from which most of the oil had been extracted, was used. The meal contained 41.5 per cent. crude protein.

*Extraction of the protein.*

An attempt was made to extract the protein by means of 0.2 per cent. caustic soda solution but was abandoned owing to the fact that it turned the meal into a gelatinous mass from which the extract could only be pressed with difficulty, and that, on acidifying this little quantity of extract, the gelatinous precipitate formed could not be granulated.

Consequently, 10 per cent. sodium chloride solution was used, 12 hours'

extraction being employed, the ensuing extract being centrifuged and filtered. In this way 8 litres of clear extract were obtained. Gradual saturation of the extract with ammonium sulphate proved that there was only one globulin present which was confirmed by a coagulation point determination. The globulin was precipitated from solution in 10 per cent. sodium chloride by 0.30–0.35 saturation with ammonium sulphate and coagulated in the same strength of sodium chloride solution at 61°. No trace of albumin was found present. This proved that the meal had been extracted with a warm solvent, thus denaturing the albumin and possibly another globulin. Nevertheless, the high yield of protein obtained by 10 per cent. sodium chloride extraction showed that the globulin obtained represented by far the greatest fraction of the protein and justified its study as a representative protein from rapeseed.

The protein was precipitated from solution by heat coagulation, the slightly acidified extract being heated to 70° in a water bath, the protein being soon precipitated as creamy white flocks. After washing several times with boiling water, the precipitate was dried by extraction for long periods with rectified spirits, absolute alcohol, and absolute ether in a Soxhlet extraction apparatus. 105 gm. of a dusty, creamy powder were obtained which was found to contain 1.87 per cent. moisture, 3.75 per cent. ash and 16.08 per cent. nitrogen (= 17.04 per cent. nitrogen calculated on a dry, ash-free basis). The preparation answered to all the protein tests. Tryptophane was found present. The small amount of ash contained calcium, sodium and chloride. The protein was undoubtedly a globulin due to (1) its solubility in salt solution, (2) the fact that it could be precipitated from solution with ammonium sulphate, and (3) its relatively high coagulation point (61°) in 10 per cent. salt solution.

A duplicate determination of the distribution of nitrogen in the protein was carried out by the van Slyke method, the average results obtained being as follows:

Table VII. *Distribution of nitrogen in colzalin, the globulin of rape seed (van Slyke method).*

	% of total nitrogen
Amide nitrogen ... ..	13.59
Humin nitrogen ... ..	3.25
Arginine nitrogen ... ..	16.39
Histidine nitrogen ... ..	12.20
Cystine nitrogen ... ..	1.04
Lysine nitrogen ... ..	2.54
Amino nitrogen of filtrate ...	43.24
Non-amino nitrogen of filtrate	8.02
Total nitrogen accounted for	100.37

From the results in Table VII the following percentages of diamino acids and ammonia were calculated.

Table VIII. *Percentages of diamino acids and ammonia in colzalin (calculated from Table VII).*

	%
Arginine	8.9
Histidine	7.8
Cystine	1.6
Lysine	2.3
Ammonia	2.9
Total	23.5

The diamino acids were determined by the method of Kossel and Kutscher (*vide* modification by Osborne, etc. [1908]) by using about 40 gm. of the protein, the following results being obtained.

Table IX. *Percentages of diamino acids in colzalin determined by the Kossel and Kutscher method.*

	%
Arginine	6.29
Histidine	3.07
Lysine	4.92*
Ammonia	2.39
Total	16.67

\* This result is calculated on the amount of picrate isolated. The amount of lysine calculated from the nitrogen precipitated by phosphotungstic acid was 7.39 per cent.

Comparing the nitrogen distribution of colzalin with the average nitrogen distribution of the soluble protoplasmic proteins of brassica plants, a close similarity, with the exception of amide nitrogen, can be observed. The protein, as it gets stored in the seed, is highly amidised. The diamino acids account for a third of the nitrogen of the proteins and arginine itself accounts for roughly a sixth. It is apparent that the quantities of the diamino acids do not suffer a change when the protein gets stored, and that an analysis of a seed protein would give a fair indication of the diamino acid content of the protoplasmic protein of the same species.

The differences in values obtained for the determination of the diamino acids by the two methods used is of general occurrence and arises mainly from the fact that what is precipitated by phosphotungstic acid does not consist only of diamino acids.

During the extraction of the globulin, owing to the gelatinous nature of the meal, there was a large quantity of salt solution still left in the wet meal and the extraction of the protein was by no means carried to its fullest extent. Nevertheless 25 per cent. of the protein in the meal was extracted by the above process. All the digestible protein in rapeseed



meal is of a composition very similar to that of the colzalin isolated, hence, when feeding with this meal it is certain that the protein added contributes at least 5 per cent. of its weight as arginine, 2.5 per cent. as histidine and 4 per cent. as lysine.

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## THE PROTEINS OF GREEN FORAGE PLANTS.

### IV. THE PROTEINS OF SOME PLANTS OF THE NATURAL ORDER UMBELLIFERAE.

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THE plants worked on in this Natural Order were the Carrot (*Daucus carota* L.) and the Parsnip (*Peucedanum sativum* Benth.). Both these plants store up reserve food in their thickened root and hypocotyl, the disposition of the tissues being similar in both "roots." For the extractions of their proteins, members of "races" of these plants containing the minimum amount of core and fleshiest annuli of bast were taken, healthy, uniformly well-grown specimens being selected.

The method of extraction of the protein was similar to that adopted for other fleshy vegetable tissue. The roots were finely minced and the juice at once pressed out (59 per cent. yield by weight of juice) and filtered by suction through a pad of cotton wool. The press-cake was stirred into a paste with water, again finely minced and again pressed, the extract being treated in the same way as the first juice. The press-cake was then treated with ether-saturated water and, after allowing to stand for half an hour, the liquid was pressed out and filtered. These three extracts were heated individually to 70° after the addition of some sodium chloride solution and a few drops of acetic acid and the coagula thus obtained were united. After washing several times with hot water during which the flocculent coagulum from the parsnip gradually became more granular, the precipitates were collected in the reservoirs of a battery of Soxhlet extractors and extracted exhaustively with rectified spirits, absolute alcohol and ether. These solvents extracted very large amounts of colouring matter (carotin) from the carrot preparation and a small amount of brown colouring matter from the parsnip. The excess ether was sucked off and the solids powdered in warm mortars and again extracted with ether for a further period of three hours, after which the excess ether was again sucked off and the preparations isolated as fine dusty powders. These were analysed after remaining in a desiccator over concentrated sulphuric acid for 48 hours (Table I).

Attempts were made to extract more protein from the residual press-cakes after the extraction of the above preparations, but acidification of the warm alcoholic alkaline extracts yielded no appreciable

precipitate in either case. The amount of residual nitrogen in each press-cake was then determined and a balance of the nitrogen made out.

Table I. *Analyses of preparations of proteins from some umbelliferous forage plants.*

Source	Moisture %	Ash %	Nitrogen %	Nitrogen (in dry, ash-free product) %
Carrot	17.44	6.42	11.01	14.72
Parsnip	15.33	6.93	9.39	12.12

The following table (Table II) shows how the nitrogen was distributed during the extraction of the proteins.

Table II. *Distributions of nitrogen in carrot and parsnip roots.*

Source of protein	Total nitrogen extracted %	Nitrogen isolated as protein %	Amide nitrogen %	Other nitrogen %	Nitrogen unextracted %
Carrot	90	36	7.3	47.7	10
Parsnip	90	34	8.0	48.0	10

The amide nitrogen was determined by Sacchse's method.

From the above table it can be seen that the percentage of nitrogen extracted was the same in both cases, and that the nitrogens entering into coagulable proteins were about the same, whereas there was also a similarity in the amounts of amide nitrogens in the juices. The plants therefore show a close similarity in their distributions of nitrogen as far as they have been investigated in this work of extraction.

The samples of proteins obtained showed all the characteristic reactions of proteins, giving all the colour tests pronouncedly. Both

Table III. *Distribution of nitrogen in proteins from the carrot and the parsnip (van Slyke)*

	Carrot protein %	Parsnip protein %
Amide nitrogen	6.47	7.34
Humin nitrogen	5.62	5.81
Arginine nitrogen	15.38	10.80
Histidine nitrogen	4.37	11.55
Cystine nitrogen	1.07	0.88
Lysine nitrogen	6.32	7.55
Amino nitrogen of filtrate	50.58	45.10
Non-amino nitrogen of filtrate	9.91	10.75
Total nitrogen accounted for	99.72	99.78

The nitrogen figures are corrected for the solubilities of the phosphotungstates of the diamino acids.

gave the coloured ring for tryptophane very prominently. They burned on ignition with the characteristic smell of burning proteins and left small amounts of white fluffy ash which contained calcium, magnesium,

sulphate and phosphate with traces of iron and chloride. The presence of a trace of carbohydrate in both preparations was detected by Molisch's test.

Duplicate determinations of the distribution of the nitrogens in these proteins were carried out by the van Slyke method, the results appearing in Table III.

From the results of Table III the proteins were found to contain the following percentages of diamino acids and ammonia.

Table IV. *Percentages of diamino acids and of ammonia in carrot and parsnip proteins.*

Amino	Carrot protein %	Parsnip protein %
Arginine	7.6	5.4
Histidine	2.6	6.8
Lysine	5.3	6.3
Cystine	1.5	1.2
Ammonia	1.3	1.4
Total protein accounted for	18.3	21.1

In both these roots a third of the nitrogen exists in the form of water-soluble protein and hence, when feeding with these roots, in the case of carrots at least 2.6 per cent. of the protein fed is as arginine, 0.9 per cent. as histidine, 1.8 per cent. as lysine and 0.5 per cent. as cystine, and in the case of parsnips, 1.8 per cent. of the crude protein fed is as arginine, 2.3 per cent. as histidine, 2.1 per cent. as lysine and 0.4 per cent. as cystine. There is, of course, a certain small amount of these amino acids present in the "other nitrogen" included in the table of distribution above (Table II), but it is not known how much or in what proportion they are present.

Certain marked differences appear in the proteins of these two plants studied. The main differences are (1) the carrot protein contains a larger percentage of monamino acids having the nitrogen present as  $\text{NH}_2$ , whereas in the parsnip protein there is a larger percentage of proline and oxyproline; (2) the carrot protein has a higher percentage of arginine and a lower percentage of histidine than the parsnip protein, but the sums of the percentages of these two amino acids in both proteins are roughly the same. No difference in nutritive value can be attributed to this difference, since arginine and histidine are interchangeable as regards physiological effect and nutritive values (Ackroyd and Hopkins, 1916).

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# THE SOLUBILITY OF SOME LIKELY SPRAY SUBSTANCES IN SOLVENTS CONTAINING SOAP. THE PREPARATION OF SPRAYING EMULSIONS.

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In previous papers it has been shown that clear solutions containing large relative amounts of paraffin oil can be prepared<sup>(1)</sup>, the solution being effected by means of cresylic acid in the presence of soap. In the present experiments phenol itself and hydrogenated phenols have also been used; solution of such substances as coal tar creosote, filtered anthracene oil ("green oil"), benzene, and bases such as pyridine and aniline, has been attempted.

## MATERIALS USED.

*Paraffin oil.* This was a "best" lighting oil bought at a local pharmacy. It was purified by shaking repeatedly with fresh lots of concentrated sulphuric acid until finally the acid assumed a straw-coloured tint only. The oil was then washed with dilute sodium hydroxide solution, then with distilled water, dried over anhydrous potassium carbonate and fractionally distilled.

A lighting oil should boil at 150°–300° C.; Pickering states that, to be an effective insecticide, paraffin oil should contain at least 40 per cent. boiling above 250° C.; Moore and Graham also point out that the high-boiling compounds of kerosene (paraffin oil) are the most toxic as contact insecticides<sup>(2)</sup>. By fractionally distilling the oil valuable information regarding the insecticidal properties of a lighting oil was obtained:

Temp. (° C.)	% by vol. of oil distilling
<145	0.69
145–180	30.04
180–215	36.90
215–250	20.94
250–300	9.80
>300 (residue)	1.63
	} = 11.43

Two gallons of oil were distilled, so that the above figures are quite significant.

The oil thus conformed admirably to the standard desirable in a

lighting oil, but not to that expected of a spraying oil, for 11.43 per cent. only boiled over  $250^{\circ}\text{C}$ . It is therefore probable that paraffin (lighting) oil from which the lower fractions have been removed would be an improved insecticide; indeed, this fact may explain the successful use of lubricating oils—higher fractions of petroleum than the lighting oils—as constituents of insecticides in America.

For the following experiments a mixture of the fractions boiling between  $145^{\circ}$ – $300^{\circ}\text{C}$ . of specific gravity  $D_{40}^{20} = 0.7884$  was used.

*Benzene*. A sample purified in the usual manner boiling at  $80.35^{\circ}$ – $80.5^{\circ}\text{C}$ .

*Pyridine*. Labelled “B.D.H. pure.”

*Aniline*. B.D.H. redistilled.

*Filtered anthracene oil*. B.D.H. sample, specific gravity  $D_{40}^{20} = 1.100$ .

*Coal tar creosote*. B.D.H. sample, specific gravity  $D_{40}^{20} = 1.005$ .

*Phenols and Hydrogenated Phenols*:

(1) *Phenol* ( $\text{C}_6\text{H}_5\text{OH}$ ). B.D.H. liquid sample (liquefied *B.P.*).

(2) *Cresylic acid* (mixture of *o*-, *m*- and *p*- cresols,  $\text{C}_6\text{H}_4\cdot\text{CH}_3\cdot\text{OH}$ ). B.D.H. pure sample.

(3) *Cyclohexanol or hexalin* ( $\text{C}_6\text{H}_{11}\text{OH}$ ). Obtained by the hydrogenation of phenol.

(4) *Methyl-hexalin* ( $\text{C}_6\text{H}_{10}\cdot\text{CH}_3\cdot\text{OH}$ ). Obtained by hydrogenation of the cresols.

*Soap*. The soft soap used was a comparatively pure sample of potassium oleate made by grinding together the calculated amount of “frozen out” oleic acid with a concentrated solution of potassium hydroxide and then heating to a melt with constant stirring. The soap, on storage, contained 37.24 per cent. of moisture<sup>1</sup>.

*Water*. Distilled water was used so as not to mask the solubility of the oils by precipitation of insoluble soaps, as would sometimes occur in practice; a fine suspension of these soaps—and of acid soaps obtained by great dilution—is liable to be confounded with a very dilute emulsion otherwise.

A very hard water would make the use of a solution of oil, where a balance of oil—phenol—soap is necessary within certain limits, impossible. Hard waters also interfere with wetting and spreading, and with emulsification when soap is the emulsifier. With reference to this

<sup>1</sup> It has been noticed in experimenting with different soaps that the capacity of aiding solution of oils depends on the type or composition of the soap. To be sure of obtaining always the same capacity for aiding solution of oils, a standard soap should be used; many spray manufacturers make such a standard product, *e.g.* potassium oleate.

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last point, Petherbridge and Dillon-Weston(3) have observed that in the Wisbech area hard water makes the formation of emulsions of certain proprietary coal tar washes containing soap as emulsifier very difficult and often impossible, gelatine, glue or size having to be added as an emulsifying agent.

### EXPERIMENTAL.

It was first necessary to ascertain the results of the addition of soap or phenol, or both, on the solubility of the chosen substances in water. Because of the practical difficulties attending the analysis of solutions containing hydrocarbons, phenols and bases, and because of the large number of determinations made (many results will not be cited), a "synthetic" method of determining solubility at room temperature was used: the substance was titrated into a definite volume of the solvent (water, soap solution, or soap solution plus phenol) until turbidity persisted on continuous shaking, signifying that further additions of the substance were being emulsified and not dissolved.

Where soap solutions were the solvent, the soapy turbidity prevented the determination of solubilities of the hydrocarbon oils but, with the concentrations of soap solutions used, the amounts of these oils completely soluble without any emulsification taking place are no doubt very small. In the cases of substances such as phenols and pyridine, addition of small amounts cleared the soap solutions to bright sparkling liquids, subsequent clouding due to excess of solute being very sharp and definite. A few instances were noticed where turbidity persisted for a few minutes even with constant agitation before solution of the solute occurred, and, consequently, in order to arrive at the correct endpoint, the solute had to be added one drop at a time with long shaking between each addition.

Table I indicates that the presence of soap in the solvent greatly increases the solubilities of phenol, cresylic acid, hexalin, methyl-hexalin and aniline, pyridine apparently being miscible in all proportions with water and the soap solutions used. The more concentrated the soap solution, the greater the solubility of the phenols, hydrogenated phenols and aniline; in the case of the hydroxyl compounds, doubling the concentration of the soap in the solvent means that more than twice as much solute liquid is dissolved by a given volume of solvent.

Table II contains the solubilities of the substances in mixtures of soap solutions and phenols or hydrogenated phenols or pyridine. A definite volume (in excess of that required to clear the soapy turbidity)

of the hydroxyl compound or of pyridine was added to a definite volume of soap solution and the solute titrated in until turbidity persisted on continuous shaking. More of the hydroxyl compound or of pyridine was

Table I.

Solvent liquid	Solute liquid	c.c. of solvent taken	c.c. of solute dissolved before turbidity*
Water†	Phenol	100	8.18
"	Cresylic acid	100	1.50
"	Hexalin	100	0.50
"	Methylhexalin	100	0.15
"	Pyridine	100	∞
"	Aniline	100	3.00
Soap solution (5%)‡	Phenol	100	57.00
"	Cresylic acid	100	9.55
"	Hexalin	100	14.25
"	Methylhexalin	100	9.75
"	Pyridine	100	∞
"	Aniline	100	6.80
Soap solution (10%)‡	Phenol	100	> 100
"	Cresylic acid	100	21§
"	Hexalin	100	36.50
"	Methylhexalin	100	30.60
"	Pyridine	100	∞
"	Aniline	100	10.50

\* This column represents the maximum amount of solute *completely soluble* (without any emulsification); it is possible, in view of Pickering's work, that this amount is not the maximum amount of solute soluble under any conditions, *e.g.* when emulsification of solute is simultaneously possible and when the initial amount of solute present is varied (4 and 1).

† The solubilities of linseed oil, olive oil, oleic acid, benzene, paraffin oil, anthracene oil, coal tar creosote, and aniline in aqueous solutions of phenol, cresylic acid, hexalin, methylhexalin and pyridine have been investigated. Inappreciable solution was obtained except in the following two cases: benzene and aniline in pyridine-water mixtures (the presence of phenols depressed the solubility of aniline in water).

The first case, the solubility of benzene in pyridine-water mixtures, has been fully investigated at 25° C. by Woodman and Corbet (5); the system was found to constitute an example of a pair of partly miscible liquids, the proportions of water, pyridine and benzene forming one phase systems being obtainable from the equilibrium diagram.

The solubilities of aniline in certain pyridine-water mixtures are as follows:

Solvent	Solute	c.c. solute soluble before turbidity
100 c.c. water + 1 8 18 20 28 40 Aniline 3.24 turbid 5.32 5.64 8.82 14.66 pyridine (c.c.)		

The meaning of the table and of the continuous titration will be found by reference to the explanation concerning Table II (this paper). It is noticeable that over 8 c.c. of pyridine must be added before the solubility of aniline appreciably increases.

‡ The concentrations refer to anhydrous potassium oleate in Tables I and II.

§ Approximate value; difficult to judge owing to formation of jellies and mucilages (1).

now added, and a new solubility determined for this extra amount by continuing the titration. The process was sometimes repeated until



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further addition of hydroxyl compound or pyridine failed to clear the turbidity (usually intensifying it), and thus one titration was made to yield a few solubilities.

Table II.

Solvent					Solute	c.c. solute soluble before turbidity			
100 c.c. 5% soap solution + phenol (c.c.)	10	18	28	—	Paraffin oil	2-10	4-36	turbid	—
"	28	—	—	—	"	2-90	—	—	—
"	10	18	28	—	Benzene	4-26	5-06	turbid	—
"	10	18	28	—	Coal tar creosote	0-70	2-54	"	—
"	10	18	28	40	Anthracene oil	0-64	2-14	2-84	turbid
"	10	18	—	—	Aniline	1-88	turbid	—	—
100 c.c. 5% soap solution + cresylic acid (c.c.)	8	—	—	—	Paraffin oil	0-50	—	—	—
"	8	—	—	—	Benzene	0-24	—	—	—
"	8	—	—	—	Coal tar creosote	turbid	—	—	—
"	8	—	—	—	Anthracene oil	"	—	—	—
"	8	—	—	—	Aniline	0-68	—	—	—
100 c.c. 5% soap solution + hexalin (c.c.)	8	10	—	—	Paraffin oil	2-82	4-68	—	—
"	8	10	—	—	Benzene	4-02	4-74	—	—
"	8	—	—	—	Coal tar creosote	1-20	—	—	—
"	8	—	—	—	Anthracene oil	1-30	—	—	—
"	8	—	—	—	Aniline	5-12	—	—	—
100 c.c. 5% soap solution + methylhexalin (c.c.)	8	—	—	—	Paraffin oil	0-20	—	—	—
"	8	—	—	—	Benzene	2-20	—	—	—
"	8	—	—	—	Coal tar creosote	turbid	—	—	—
"	8	—	—	—	Anthracene oil	"	—	—	—
"	8	—	—	—	Aniline	2-00	—	—	—
100 c.c. 5% soap solution + pyridine (c.c.)	8	—	—	—	Paraffin oil	turbid	—	—	—
"	8	—	—	—	Benzene	2-08	—	—	—
"	8	10	18	28	Aniline	7-34	7-52	10-32	17-82
"	40	60	—	—	"	28-00	45-00	—	—
"	8	—	—	—	Coal tar creosote	turbid	—	—	—
"	8	—	—	—	Anthracene oil	turbid	—	—	—
"	8	10	18	28	Oleic acid*	1-64	2-24	5-94	11-28

\* Linseed and olive oils were found to be insoluble in 100 c.c. 5 per cent. soap solution plus 8 c.c. of pyridine; these two true oils, and oleic acid, were experimented on because of the insecticidal value of such fatty substances (6 a).

The sub-columns under the heading "solvent" indicate the volume of the hydroxyl compound or pyridine added previous to titration. This volume was always sufficient to clear the soap solution but never in excess of the amount indicated by Table I (*i.e.* the limit of solubility of the added substance was never exceeded). The figures in these sub-columns, reading horizontally, or bracketed as in the case of pyridine, represent the continuous titration method already discussed, and the corresponding figures in the sub-columns of the last column are the solubilities of the solute so found.

A "turbid" in the first sub-column of the last column indicates

insolubility, though, if less of the hydroxyl compound or pyridine had been added, a solubility might have resulted. This is well brought out in the case of the solubility of paraffin oil in phenol-soap solution (first two results, Table II). By the continuous titration method discussed, it was found that 100 c.c. of 5 per cent. soap solution plus 18 c.c. of phenol dissolved 4.36 c.c. of paraffin oil before turbidity resulted; addition of phenol to make a total of 28 c.c. intensified the turbidity already existing, so that the oil was now less soluble. This was shown by adding 28 c.c. of phenol to 100 c.c. of the soap solution and determining the solubility of the oil (2.90 c.c.). The conclusion is, that though 100 c.c. of 5 per cent. soap solution dissolve 57 c.c. of phenol (Table I), they are capable of dissolving only 18 c.c. of phenol plus 4.36 c.c. of paraffin oil, or 28 c.c. of phenol plus 2.90 c.c. of oil; so that, though the presence of the phenol in all cases tried made the solution of paraffin oil possible to an appreciable extent, there was evidently a balance of soap, phenol and water giving an optimum solubility of oil.

It will be noticed that the addition of a phenol or hydrogenated phenol to soap solution causes appreciable solution of the oils used in general. Except in the case of cresylic acid, benzene was more soluble than paraffin oil. Hexalin and then phenol appeared the best aids to solution of paraffin oil; but here the results were liable to be misleading because of the very dilute solution (5 per cent.) of soap used; a change in the balance of soap to water would possibly have changed this order, for previous experiments have shown cresylic acid to be a very efficient aid to solution of paraffin oil(1).

The case of aniline is peculiar, for its solubility is definitely depressed by addition of the hydroxyl compounds; addition of pyridine, however, increases the solubility. A dilute pyridine-aniline spray in soap solution should prove a fairly effective contact insecticide without much scorching action on foliage for such pests as, say, capsid bug; it is also conceivable that such a spray would be partially successful as an internal poison insecticide and as an egg wash.

Foreman and Graham-Smith, in an interesting pamphlet on the prevention of nuisances arising from flies and putrefaction(7), have investigated very fully the toxic action of such substances as aniline, pyridine and creosote oil (coal tar) on flies, maggots and eggs. Solutions and emulsions of aniline are quoted which are toxic to adult flies either when taken by the mouth or by actual contact; dilute solutions of aniline in water and emulsions in soft soap solution were also found effective against fly-maggots and eggs.

Tattersfield and Roberts, in a comprehensive survey of the toxicity of the vapours of organic substances to the very resistant wireworm(8), have also experimented on

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aniline and pyridine. The vapours of these two substances were stated to be of "moderate toxicity"; the full value of this statement will be appreciated when it is borne in mind that the vapour of carbon disulphide—essentially a soil fumigant—was, in parallel experiments, demonstrated to possess a much lower toxic effect to wireworms than the vapours of either aniline or pyridine.

In these circumstances the solubilities of aniline in mixtures of 10 per cent. potassium oleate solution, plus various likely spray substances, might be of use. The solubilities were determined by the author by the continuous titration method outlined above:

Solvent					Solute	c.c. solute soluble before turbidity			
100 c.c. 10 % soap solution	3	5	6	8	Aniline	9.8	9.8	9.8	10.0
+ pyridine (c.c.)	18	20	30	40		13.8	15.0	22.5	32.1
100 c.c. 10 % soap solution	2	3	6	8	,,	9.6	9.7	9.7	9.8
+ 2 c.c. of hexalin									
+ pyridine (c.c.)	2	3	6	8	,,	9.5	turbid	9.6	9.7
100 c.c. 10 % soap solution	10	—	—	—		10.0	—	—	—
+ 4 c.c. of hexalin					,,				
+ pyridine (c.c.)	2	15	—	—		7.0	9.2	—	—
100 c.c. 10 % soap solution	2	—	—	—	,,	4.7	—	—	—
+ 2 c.c. phenol									
+ pyridine (c.c.)	3	5	6	8	,,	6.0	turbid	turbid	turbid
100 c.c. 10 % soap solution	5*	6	8	—		1.8	turbid	turbid	—
+ 4 c.c. cresols									
+ pyridine (c.c.)									

\* Thick and turbid with entangled air when less pyridine present.

The table shows that there must be at least 8 c.c. of pyridine present with a 10 per cent. potassium oleate solution in order to dissolve the same amount of aniline as is dissolved by the soap solution alone; lesser amounts depress the solubility of aniline slightly; larger amounts raise the solubility. Hexalin has apparently no action on the solubility of aniline when pyridine is also present, but in like circumstances phenol and creylic acid further depress the solubilities.

The scope of the experiments already described was now extended; known amounts of potassium oleate (containing 37.24 per cent. of moisture) and of the phenol or hydrogenated phenol were placed in stoppered graduated cylinders and subjected to hand-shaking. The character and volumes of the resulting mixtures were noted, as also the behaviour on dilution with definite amounts of water.

5 c.c. of a mixture were taken and a definite amount of oil added from a standard burette; the resulting mixture was made up to certain dilutions with water to see if the mixture were *totally* soluble or not. By varying the amounts of oil added initially to the 5 c.c. of soap-phenol mixture and repeating the process, the amount of oil completely soluble

on subsequent dilution was got within limits by a method of trial and error. Table III gives the average of two nearly equal volumes of oil, one of which is, and one of which is not, completely soluble on subsequent dilution; the ranges of dilution at which solubility occurred are also noted in Tables III and IV; thus no distinguishing letter means the whole mixture was soluble when made up to 50 c.c. with water; (a), (b), (c) and (d) mean that the ranges of dilution where complete solubility occurred are respectively 50-100 c.c., 100-250 c.c., 250-500 c.c. and 500-1000 c.c.

Table III.

Name of mixture	gm. of "moist" soap to c.c. of hydroxyl-compound in mixture	Character of mixture	Volume of mixture (c.c.)	Dilution of 5 c.c. of mixture gives	c.c. of benzene dissolved by aid of 5 c.c.	c.c. of paraffin oil dissolved by aid of 5 c.c.	c.c. of anthracene oil dissolved by aid of 5 c.c.	c.c. of coal tar creosote dissolved by aid of 5 c.c.
Phenol A	100 : 200	Solution	299	Solutions	1.31	1.12	0.12	0.47
" B	100 : 150	"	249	"	1.62	1.37	0.37	0.62
" C	100 : 100	"	199	"	1.87	3.12	0.37	0.75
" D	100 : 50	Mixture	—	—	—	—	—	—
Cresols A	100 : 200	Solution	300	Solutions (b)	—	0.37 (d)	0.12	0.39 (d)
" B	100 : 150	"	249	" (a)	—	1.12 (d)	0.12	0.40 (d)
" C	100 : 100	"	199	Solutions	—	7.12 (a)	0.12	0.40 (d)
" D	100 : 75	"	174	"	—	5.35 (a)	0.20	0.37 (d)
" E	100 : 50	Mixture	—	—	—	—	—	—
Hexalin A	100 : 200	Solution	300	Solutions	—	0.37	0.12	0.18
" B	100 : 150	"	250	"	—	1.87	0.37	0.62
" C	100 : 100	"	199	"	—	3.87	0.87	1.12
" D	100 : 75	"	174	"	—	4.87	0.87	0.90
Methyl-hexalin A	100 : 200	"	300	Emulsions	—	0.12	0.12	0.12
" B	100 : 150	"	250	Solutions (a)	—	2.37	0.12	0.12
" C	100 : 100	"	198	Solutions	—	2.67	0.12	0.20
" D	100 : 75	Mixture	—	—	—	—	—	—

It will be seen that the best substance for admixture with potassium oleate containing 37.24 per cent. of moisture for the purpose of aiding solution of paraffin oil is the mixture of cresols known as cresylic acid, the next best being hexalin. Solution of anthracene oil and coal tar creosote can be obtained to a limited extent, particularly with the use of hexalin, but the volume of these oils completely dissolved are relatively small compared with the amount of hexalin necessary. It may thus be stated that the preparation of *solutions* of coal tar fractions would be too dear in practice; these must be applied as emulsions, particularly as the so-called "miscible oils" now on the market<sup>1</sup>.

In performing these later experiments the soap was mixed with the phenol or hydrogenated phenol firstly; because of subsequent ease in

<sup>1</sup> See later portion of paper for explanation of "miscible oils."

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solution on dilution and, secondly, because of a possible complication which might have arisen: Pickering has shown that the volume of oil dissolved by soap solutions containing a constant amount of soap depends on two factors(4), (a) the concentration of the soap solution, a larger bulk of water meaning solution of less oil, and (b) the amount of oil initially present, increase in this amount increasing subsequent solution. Thus his results for a potash soap and a lighting oil:

Oil taken	Oil dissolved (c.c.) by 100 gm. of anhydrous soap diluted with water					
	100 c.c.	200 c.c.	300 c.c.	500 c.c.	900 c.c.	1900 c.c.
33	29.4	21.8	18.6	13.0	3.2	3.6
80	53.2	23.2	19.4	5.6	2.8	5.4

demonstrate as clearly as can be expected in work of this type that these factors hold pretty generally, the oil which is not dissolved being emulsified. As one of the chief aims in the present work has been to measure the volumes of oil completely soluble on subsequent dilution without entailing any emulsification, it will be seen that factor (b) has been avoided. Not so factor (a).

A new series of experiments was made to test the effect of factor (a): to 5 c.c. of the soap-hydroxyl compound mixtures (see Table III) water was added in definite quantity. The amount of oil soluble in the resulting

Table IV.

Name of mixture	Amount taken (c.c.)	Amount of water added (c.c.)	Description of resulting mixture	Amount of paraffin oil totally soluble on subsequent dilution (c.c.)
Phenol C	5	0	Clear solution	3.12
"	5	1	"	3.05
"	5	2.5	"	3.12
"	5	5	"	3.12
"	5	45	"	<1
Cresols C	5	0	"	7.12 (a)
"	5	1	"	6.87 (b)
"	5	2.5	"	6.87
"	5	5	Cloudy and stiff	7.12
"	5	45	Clear solution	<2
Hexalin C	5	0	"	3.87
"	5	1	"	3.70
"	5	2.5	"	3.85
"	5	5	"	3.86
"	5	45	"	<1
Methylhexalin C	5	0	"	2.67
"	5	1	Cloudy	2.75
"	5	2.5	Cloudy thin mucilage	2.95
"	5	5	Cloudy thick mucilage	2.12
"	5	45	Clear solution	<1

mixtures was now determined by the method of trial and error previously described, the results being quoted in Table IV.

The mixtures used all contained 2.5 gm. of potassium oleate (containing 37.24 per cent. of moisture) in 5 c.c. (see Table III). Previous dilution of these mixtures with equal volumes of water (so that 1.57 gm. of anhydrous soap was now present to about 6 c.c. of water) did not affect very greatly the amount of paraffin oil capable of being put in solution; this is quite a different result from that of Pickering's with soap solution (*loc. cit.*); much greater dilution of the mixtures, however, previous to admixture with the oil, makes them less efficient as oil solvents. Factor (a) plays a restricted rôle, therefore, when phenols or hydrogenated phenols are added to the soap.

#### USE OF OIL SOLUTIONS AND EMULSIONS AS SPRAY FLUIDS.

Dilute paraffin oil solutions and non-creaming emulsions<sup>1</sup> made by the aid of cresols-soft soap solutions have been advocated on theoretical grounds as foliage contact insecticides(1). Now Fleischer's results with respect to the scorching effects of "Sapocarbolis," "Creolines" and "Lysols" on leaves(6b) show that the inclusion of cresylic acid in a spray is dangerous, as the author's results also tend to show(1); for this reason it would probably be preferable to use hydrogenated phenols (known to have decided insecticidal properties<sup>2</sup>), in virtue of their lack of phenolic and corrosive properties(9). If, on the grounds of *present*<sup>3</sup> expense and greater effectiveness as an aid to solution of paraffin oil, cresylic acid be preferred, then a dilute non-creaming emulsion might be used instead of a dilute solution containing the same amount of oil, as the amount of cresylic acid necessary for such an emulsion need only be small<sup>4</sup>.

<sup>1</sup> In a previous paper this non-creaming or slow-creaming of emulsions has been put down to equalisation of the densities of the phases by the cresylic acid, which is more miscible with the oil than the aqueous phase (1). Consideration of the relative amounts of oil, soap solution and phenol present, and of the densities (paraffin oil = 0.7884, cresylic acid = 1.05 and soap solution = 1.0 (approx.)) show that the amount of the phenol present is too small to make the above the only reason; the actual solution of oil by the aqueous external soap phase must also have a great effect on the relative densities of the phases. These slow-creaming emulsions, therefore, must contain large amounts of oil in actual solution.

<sup>2</sup> Private communication from the late Prof. Lefroy.

<sup>3</sup> The writer has no definite knowledge on the costs of hydrogenated phenols (procured from R. W. Greeff and Co., London); hydrogenation should, however, be cheap; and usually demand for a chemical means that it becomes much cheaper.

<sup>4</sup> Solutions of oil, soap and phenol (or hydrogenated phenol) would be so concentrated as to be economically transported.

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It has been mentioned previously that actual solution of the heavier fractions of coal tar such as creosote and anthracene oil will not be accomplished to such an extent as to give winter or summer washes. The only alternative, therefore, is the preparation of suitable emulsions by the easiest methods.

### PREPARATION OF SPRAYING EMULSIONS.

The confusion which exists in the minds of many people with regard to the difference between emulsions and solutions has given rise lately to a new term—"miscible oils." To a chemist, the false nature of the term is at once apparent, for oils and water are *immiscible*; yet advertisements and papers are continually being published which show little appreciation of the true meaning of the word "miscible." If an oil is made up in such a way with other substances that, on pouring into water, *mixing* occurs easily, the oil is wrongly said to be miscible, though *solution* has not occurred. The truth is, of course, that an emulsion has been formed in an easy manner without shaking.

Bourcart (6 c), evidently quoting from a U.S. Government bulletin, states that oil sprays "are made from either miscible oils or emulsion stocks. A miscible oil is an oil solution of an emulsifier. An emulsion stock is a concentrated emulsion...." This statement is quite correct except for the use of the term "miscible oil," the oil merely being miscible with the emulsifier before addition of water.

The only cases in which an oil may be truly termed "miscible" are those such as are described in the present and preceding papers (1), where the oil, in solution with emulsifier and phenol or hydrogenated phenol, is totally *dissolved* on pouring into water.

The truth is that the materials for making a dilute emulsion can be got together in two ways: a concentrated stock emulsion of oil in a concentrated solution (or pseudo-solution) of emulsifier such as soap, caseinate, etc., can be made by agitation or shaking, and subsequently easily diluted to the required strength by merely adding water, or, the oil and emulsifier may be combined by some means to give a solution which, on dilution, gives the required emulsion (so-called "miscible oil"). Among these latter are included the proprietary coal tar washes which are supposed to give stable emulsions by stirring into water.

Two problems thus arise: in the first place, ease of emulsification in preparing a concentrated stock emulsion is desirable and, secondly, the so-called "miscible oil" should mix easily—ideally by pouring into and

merely stirring—with water to give the required emulsion; too often these “miscible oils” are very difficult of dilution to dilute emulsions.

The first problem is remarkably complex. With oils such as paraffin it is comparatively easy to form, by agitating, pumping or shaking apparatus commonly met with in works, a suitable concentrated stock emulsion in soap solution; the heavier oils such as the coal tar oils are difficultly emulsifiable in small quantity even in the laboratory. It has been found that adding the oil to the emulsifier solution in small quantities at a time, emulsifying between each addition, together with intermittent agitation, facilitates the formation of concentrated emulsions.

The choice of emulsifier must also be wise; thus soap does not tend to give stable emulsions of cresylic acid in water; and the use of gelatine will not allow the formation of stable cresylic acid in water emulsions, besides complicating matters by often giving the reverse type of emulsion<sup>(10)</sup>. In this case, therefore, some other emulsifier must be sought.

The effect of temperature on ease of emulsification is also very great, and experiments have been done with the view of ascertaining these temperature effects when using common emulsifiers.

#### EFFECT OF TEMPERATURE ON EMULSIFICATION.

The commonest substance for use with spray fluids as a wetter, spreader and emulsifier is, undoubtedly, soap. Lately, however, there has been some tendency for chemists and plant pathologists to experiment with gelatine (sold commercially in the cheap and more soluble forms of powdered glue, fish glue, size, etc.). Thus gelatine has been proved to augment the amount of spray retained by foliage<sup>(11)</sup>; it is the best protective colloid for lead arsenate suspensions<sup>(12)</sup>; its effect on a mixed lime sulphur-lead arsenate spray has been tested<sup>(13)</sup>; and, in practical trials with oil emulsions as winter washes, gelatine was found to be much superior to any other emulsifier in the case of certain abnormally hard waters occurring in the Wisbech district<sup>(3)</sup>.

In these circumstances it is important to know the effect of temperature on emulsification when using either soap or gelatine as emulsifiers, and the present experiments deal with this aspect of emulsification.

The system chosen was toluene-water-emulsifier, the emulsifier being comparatively pure anhydrous sodium oleate in some experiments, and anhydrous gelatine, purified by a modification of Loeb's process, in others<sup>(14)</sup>.

The method adopted for making the toluene emulsions was the intermittent shaking method of Briggs<sup>(15)</sup>; this method has been stan-



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dardised in previous experiments(16). It was first necessary to find by trial the concentrations of emulsifier which would give significant results, and finally 0·3 per cent. gelatine and 0·4 per cent. sodium oleate solutions were adopted.

The emulsifier solutions were kept at room temperature; as occasion demanded, 10 c.c. were pipetted into a stoppered 50 c.c. cylinder and 20 c.c. of pure toluene were then added in such a manner as not to disturb the aqueous medium. The cylinder was placed in a thermostat at the desired temperature for 30 min., stoppered, rapidly withdrawn and given a complete shake (up and down motion of the hand), and then quickly replaced in the thermostat. The cylinder was withdrawn at the end of every 30 sec., and given a shake (the time the cylinder was out of the thermostat being 2–3 sec. in every 30 sec.), until complete emulsification occurred.

As the properties (and therefore the number of shakes necessary to form a standard emulsion) of a gelatine solution are known to vary greatly with the age of the solution (16), two sets of results were obtained in the case of gelatine, the gelatine solution being kept in one case 5 hrs., and in the other case 5 days, at room temperature, previous to experiment.

The results are given in the following table:

		Emulsifier 0·3% gelatine			
		Gelatine solution 5 hrs. old		Gelatine solution 5 days old	
Emulsifier 0·4% Na oleate					
Temp. of Thermo- stat (°C.)	No. of 30 sec. shakes	Temp. of Thermo- stat (°C.)	No. of 30 sec. shakes	Temp. of Thermo- stat (°C.)	No. of 30 sec. shakes
21	145	23	14	19	22
31	114	29	12	27	12
41	50	39	20	36	15
51	12	49	21	47	22
61	5	59	20	54	22

Another experiment was carried out on the four days old solution of gelatine. The number of 30 sec. shakes then required to form the standard emulsion mixture at 17° C. was 22; on heating the solution to 80° C. and allowing to cool for 7 hrs. at 17° C., the number of shakes now required was 15. The blue opalescence of the aged solution disappeared on heating, the cooled solution being noticeably less viscous.

Shaking the emulsifier solutions previous to any of the above experi-

ments did not materially alter the number of shakes subsequently necessary.

The results indicate a great difference in the effects of temperature on emulsification when using soap and gelatine as emulsifiers. The sprayer would obviously be well advised to make concentrated soap emulsions, for subsequent dilution, at a temperature of about 60° C.; when using gelatine as emulsifier, the temperature should be about 25–30° C.; and, to prevent “setting,” the subsequent dilution to the concentration needed for spraying would have to be immediate.

This difference in behaviour might be accounted for as follows: *rate* of adsorption is increased by rise of temperature<sup>(17)</sup>, though the *amount* adsorbed is less, as is only to be expected, on thermo-dynamic grounds, from the negative temperature coefficient of surface energy. Thus, ordinarily, rise of temperature should facilitate the formation of emulsions, the number of shakes showing a continuous decrease. This behaviour is experienced in the case of soap, and with gelatine up to about 30° C.; beyond this temperature, however, emulsification is more difficult with gelatine. Now a fresh gelatine solution is definitely emulsoid, but the properties of an ageing solution can be best explained on the assumption of the gradual formation of a solid network structure<sup>(18)</sup>. Heating a gelatine solution to about 30° C. begins to destroy this network structure (*e.g.* a gelatine gel liquefies at this temperature approximately), and further heating continues the process; heating, therefore, may be regarded as a reversal of the ageing process, the gelatine solution beginning a new life-cycle on cooling, being definitely emulsoid.

Thus, though a gelatine solution shows normal behaviour up to about 30° C. (old solutions, where the network is definitely established, give more significant results), destruction of the solid particles necessary for emulsification<sup>(19)</sup> make the process more difficult of accomplishment above this temperature.

The reason that an aged cold solution of gelatine is not such a good emulsifying medium as one a few hours old (though more solid matter is present in the former) is probably mainly mechanical, hinging on the great increase of viscosity with age<sup>(16)</sup>, for, as previously stated, rise in temperature increases the rate of adsorption and not the amount adsorbed.

Previous shaking of the gelatine solution does not materially alter the number of shakes required for emulsification, because the network is broken down to *débris* suitable for absorption, in an exactly similar manner, by the first intermittent shake in the standard method used, the two cases then becoming identical.

## FORMATION OF MISCIBLE OILS.

The second problem of emulsification, the formation of a so-called "miscible oil" which will form an emulsion on merely stirring into water, without any difficulty or danger due to reversal of type, is a pressing one in view of the ever-increasing use of oil sprays.

The present paper and preceding ones have solved this problem as regards paraffin oil and benzene<sup>(1)</sup>, by previous admixture with soap and phenol or a hydrogenated phenol, a solution is formed which, on merely pouring into water and stirring, gives either a solution of the oil or a mixed solution and stable emulsion, according to the amount of oil present. "Cresoap," a solution of cresylic acid and fish-oil soft soap similar to the author's, is now used extensively in America as the best emulsifier for oils<sup>(6c)</sup>. Moreover, by properly balancing the constituents of the original mixture, slow-creaming of some of the emulsions of light oils may be obtained<sup>(1)</sup>.

Emulsions made in the same manner by adding coal tar creosote or anthracene oil to cresols C solution (Table III) and then adding the water, were found to be not so good as those made with paraffin oil or benzene; thus the creosote oil emulsion, though the better of the two and stable to creaming, contained tiny particles of unemulsified oil.

In conclusion it is necessary to thank Dr R. S. Morrell of Wolverhampton for drawing the writer's attention to the possibilities of the hydrogenated phenols, and to V. Heaton, Esq., B.Sc., for kindly procuring samples of the same.

## SUMMARY.

A lighting (paraffin) oil bought casually, such as is often used in paraffin emulsions for spraying, has been subjected to fractional distillation, and shown to be unsuitable as a spraying oil.

Approximate solubilities at room temperature of various "oils" in solvents such as soap solutions with and without the addition of phenols, hydrogenated phenols and pyridine, have been determined. Spray fluids containing paraffin oil, benzene, and aniline in solution are economically possible, but coal tar fractions such as anthracene and creosote oils, will, owing to lack of solubility, have to be applied to plants as emulsions. Cresylic acid is the best aid to solution of paraffin oil, but is probably more injurious to foliage than the dearer, hydrogenated phenol, hexalin.

Experiments have been made on the influence of temperature when using soap (sodium oleate) and gelatine as emulsifiers; rise in temperature is found to facilitate the formation of emulsions in soap solutions, but to have a much more complex effect when gelatine is the emulsifier. A possible explanation of these facts is given.

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# THE COMPOSITION OF FLAKED MAIZE.

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DURING recent years a growing interest has been manifested by feeders of stock in the form of cooked maize known as flaked maize, which is produced on the industrial scale by the steaming and rolling of maize grain. Following the publication of the results of an investigation into the comparative values of dry, soaked, cooked and flaked maize for pig-feeding<sup>1</sup>, in which investigation the high digestibility and feeding value of flaked maize were amply demonstrated, frequent enquiries have been received as to whether it is justifiable to assume an equal feeding value for the several brands of flaked maize which are put on the market at the present time.

With the object of settling this point, and with the co-operation of the Editor of *Farming* and the various manufacturers concerned, samples of the different brands of flaked maize have been collected during 1926 for the purposes of comparative analysis. The results are given in Table I, in which table the data are further compared with the corresponding figures for the sample of raw maize used in the Cambridge pig-feeding trials.

Table I. *Composition of Different Brands of Flaked Maize  
(on basis of dry matter).*

	A	B	C	D	E	F
	Raw maize					
	%	%	%	%	%	%
Crude protein	10.95	10.99	10.99	11.24	10.93	10.56
True protein	10.18	9.83	10.07	10.96	10.13	10.43
Ether extract	5.12	4.78	4.59	4.67	3.92	4.97
N.-free extractives	79.80	81.51	81.34	80.89	81.86	81.67
Crude Fibre	2.27	1.67	1.69	1.78	2.06	1.59
Ash	1.86	1.05	1.39	1.42	1.23	1.21
Lime, CaO	0.02	—	—	—	—	Trace
Phosphate, P <sub>2</sub> O <sub>5</sub>	0.95	0.45	0.72	0.81	0.64	0.59

B, C, D, E and F represent a number of well-known brands of flaked maize. C and D are samples of the same brand, differing, however, by the fact that C was made from a mixture of 1 part white African maize and 4 parts yellow Plate maize, whereas D was made wholly from yellow Plate maize.

<sup>1</sup> Woodman, *Journ. Agric. Sci.* **15**, 1, 1925 and *Journ. Minist. Agric.* **31**, No. 12, March, 1925.

*Comments on Table I.*

(1) Flaked maize as made by the different manufacturers at the present time is a reasonably uniform product. This is brought out by a consideration of the values obtained for the percentages of the main constituents, namely, carbohydrate and protein. Slight fluctuations in composition from sample to sample of even the same brand of flaked maize are naturally to be expected, since different consignments of raw grain will not display constancy of composition. Indeed, the differences in composition noted between the samples of flaked maize used in this work may simply be due to the fact that the brands were made from different supplies of raw grain.

(2) It is obviously the aim of the manufacturer to include the whole of the contents of the maize grain in the flaked product, and any minor differences between the composition of raw and flaked maize appear to be unavoidably incidental to the processes of steaming and rolling. The reduction in oil content during the process is not necessarily so large as was concluded from the analysis of the sample of flaked maize used in the Cambridge feeding trials. The 1923 sample contained only 2.16 per cent. of oil on the basis of dry matter, whereas the average oil content of the samples under investigation was 4.59 per cent. of the dry matter.

(3) Flaked maize appears to contain little or no lime, though this is scarcely surprising in view of the extremely low percentage of this constituent in raw maize. This fact may usefully be applied in investigations dealing with the effects of lime-deficient rations on the metabolism of animals.

(4) The moisture content of the samples displayed considerable variation, ranging from 6.61 to 14.43 per cent. This may partly be due to moisture taken up during storage and transport. It is strongly to be recommended, however, that the final process of drying the flakes should be so regulated that the moisture content *at the time of weighing into the sacks* should always have about the same value (say 10 to 11 per cent.). A 1-cwt. sack of flaked maize would then always contain the same weight of *dry* matter, and this would obviously be unaffected by moisture taken up during storage or transport.

*(Received October 27th, 1926.)*

# THE COMPOSITION OF MILK. PART I.

## VARIATION IN THE SOLIDS NOT FAT, FAT AND PROTEIN CONTENT OF COW'S MILK, AND THEIR RELATIONSHIP.

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(With Three Charts.)

VARIATION in composition is a matter of the greatest importance to all concerned in the production and marketing of vegetable and animal commodities. Milk is one of the most important products of agriculture, and, moreover, in this country, the bulk of it reaches the consumer in its natural state, *i.e.* without alteration in composition. Variability in the food value of milk is therefore a matter of great concern, and in England and many other countries the purchaser is legally protected in this respect.

In recent years dairy farmers have paid considerable attention to variations in yield and quality of milk, as instanced by the formation of numerous milk-recording societies throughout this country, but with regard to quality, fat percentages only appear to have been considered. It may perhaps be relevant to mention here that the calorific value of the non-fatty solids in a given weight of average milk is nearly 15 per cent. greater than the calorific value of the fat. This fact, combined with the knowledge that the percentage of solids not fat in mixed milk is subject to considerable variation, warrants much more attention being paid to this portion of the constituents of milk than heretofore. One frequently observes articles and papers dealing with the quality of milk in which no cognizance is taken of the fact that milk contains other solids than fat. Again, investigations into the variation in the percentage of non-fatty solids appear to have been few in number and scanty.

In view of the meagre authentic data available, and as a result of many enquiries from milk producers who have experienced trouble with regard to variability in the solids not fat content of the milk produced by their herds an investigation into this question was commenced at the Midland Agricultural and Dairy College in 1923, and the data obtained forms the basis of this and the following paper.

## OUTLINE OF INVESTIGATION.

From February, 1923 to December, 1924 the mixed milk of one herd only (No. 1) was sampled weekly (morning and evening samples) and these samples submitted to analysis. In January, 1925, it was decided to select other herds in different parts of the country, and arrangements were made to sample the milk from 14 additional herds. Two of these were commenced in January, and others in the succeeding months. Sampling in the case of 11 herds ceased in September of that year, but sampling of the other four herds was continued until the end of February, 1926.

The herds were selected so as to include as great a variation as possible as regards size of herd, climatic conditions, soil types and general management. Naturally all varying types of herd could not be included in the investigation, since the analytical assistance available was limited.

The herds selected are as follows:

Herd No.	County	Breed	No. of cows in herd during period of investigation	Soil type	General remarks
1.	Leicester	Pedigree and non-pedigree Shorthorn	30-35	Mixed. Boulder Clay, Keuper Marl, River Gravel and Alluvium	Grassland generally deficient in lime. Some abortion in herd
2.	Wiltshire	Crossbred Shorthorn and Irish	60-170	Wiltshire Downs (Chalk)	Cows lie out night and day all the year. Rationed herd. Good management
3.	Yorkshire	Pedigree Friesian	5-9	Glacial Drift overlying Chalk	Average yield 1500 gal. Balanced rations. Grassland well slagged, but some deficiency in lime
4.	South Derbyshire	Crossbred Shorthorn	25-28	Mixed. Mainly Bunter and Breccia (Permian)	Abortion prevalent. Grassland generally deficient in lime and phosphate
5.	North Derbyshire	Non-pedigree Shorthorn	10-15	Millstone Grit	Grassland generally deficient in lime. Herd not rationed
6.	North Derbyshire	Crossbred	5-8	Millstone Grit	Grassland very deficient in lime and phosphate. Herd not rationed. Typical North Derbyshire hillside farm
7.	Wiltshire	Non-pedigree Shorthorn	60-65	Wiltshire Downs (Chalk)	Cows out at grass night and day all the year



*The Composition of Milk*

Herd No.	County	Breed	No. of cows in herd during period of investigation	Soil type	General remarks
8.	Rutland	Non-pedigree Shorthorn	20-25	Boulder Clay and Middle Lias	Average yield 840 gal. Rationed herd. Good management
9.	Rutland	Non-pedigree Shorthorn	18-20	Middle Lias	Average yield 840 gal. Rationed herd. Good management. Belongs to same farmer as Herd 8
10.	Leicester	Pedigree Shorthorn	18-22	Keuper Marl	Grassland deficient in lime. No artificials used
11.	Yorkshire	Non-pedigree Shorthorn	35-40	Millstone Grit	Herd frequently changing. All cows bought in. None reared. Part of grassland deficient in lime
12.	Notts.	Non-pedigree Shorthorn	8-10	Keuper Marl and Alluvium	Average yield 650 gal. Herd not rationed
13.	Wiltshire	Crossbred Shorthorn and Irish	25-30	Wiltshire Downs (Chalk)	Farm run down. Recently taken over
14.	South Derbyshire	Pedigree British Friesian	20-25	Boulder Clay overlying Keuper Marl	Average yield 900 gal. Some grassland deficient in lime and phosphate
15.	Shropshire	Non-pedigree Shorthorn	40	Keuper Marl	Typical Shropshire cheese-making farm

With the object of endeavouring to throw more light on the nature of solids not fat variation in milk, determinations of fat, total solids, total protein and mineral constituents were made in the weekly samples of mixed milk obtained from the 15 herds selected. In this paper are given data concerning the fat, solids not fat and protein content.

*Analytical methods employed.*

Fat was determined by the Gerber test. Solids not fat was calculated from the fat and total solids, the latter being determined by evaporation. Total protein was calculated from nitrogen  $\times 6.38$ , nitrogen being estimated by the Kjeldahl method.

*Mean percentages.* Mean percentages of these constituents in samples from the individual herds are given in Table I.

Table II gives the number and percentage of samples below the presumptive standards (3 per cent. fat and 8.5 per cent. solids not fat) in each herd.

It is apparent from these tables, that deficiency in solids not fat was

not uncommon in the majority of the herds, and that it was more serious than fat deficiency. It should be mentioned here that of the 15 herds, five were known beforehand to have given samples of mixed milk below 8.5 per cent. in solids not fat. These are Nos. 1, 10, 11, 12, and 14, two

Table I.

Herd	No. of samples	Mean percentages of		
		Fat	Solids not fat	Protein
1	58	3.67	8.64	3.28
2	66	3.80	8.98	3.40
3	64	3.77	8.62	3.13
4	55	3.78	8.73	3.25
5	98	3.56	8.74	3.30
6	98	3.50	8.64	3.03
7	44	3.63	8.78	3.25
8	30	3.74	8.81	3.28
9	28	3.65	8.76	3.26
10	28	4.11	8.97	3.52
11	29	4.02	8.71	3.31
12	30	3.71	8.78	3.24
13	23	3.44	9.03	3.39
14	67	3.42	8.58	3.16
15	12	4.04	8.92	3.41

Table II.

Herd	Total no. of samples	No. of samples below		Percentage of samples below	
		3% fat	8.5% solids not fat	3% fat	8.5% solids not fat
1	58	2	13	3.4	22.4
2	66	12	4	18.2	6.0
3	64	0	15	0	23.4
4	55	2	3	3.6	5.5
5	98	8	10	8.2	10.2
6	98	9	23	9.4	23.5
7	44	11	4	25.0	9.1
8	30	0	1	0	3.3
9	28	0	0	0	0
10	28	0	0	0	0
11	29	1	3	3.4	10.3
12	30	6	2	20.0	6.7
13	23	0	0	0	0
14	67	8	27	11.9	40.0
15	12	0	0	0	0

of which gave milk above the average of the 15 herds in solids not fat content. Not the slightest suspicion was attached to the remainder of the herds, and they were selected mainly on account of great differences in soil, climatic conditions, and general management.

# The Composition of Milk

## Frequency distributions.

The frequency distributions of fat, solids not fat and protein percentages are given in Tables III, IV and V.

Table III. *Frequency distribution of fat. Mixed milk.*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
2.2-2.39	—	—	—	—	1	1	1	—	—	—	—	—	—	—	—	3
2.4-2.59	—	3	—	—	1	—	2	—	—	—	—	1	—	1	—	8
2.6-2.79	1	2	—	—	2	3	2	—	—	—	1	1	—	—	—	12
2.8-2.99	1	7	—	2	4	6	6	—	—	—	—	4	—	7	—	37
3.0-3.19	3	6	2	3	6	14	7	1	4	1	1	3	4	12	—	67
3.2-3.39	8	1	9	9	16	19	4	6	3	2	2	1	6	9	1	96
3.4-3.59	13	2	14	9	14	15	—	3	3	1	6	2	5	11	1	99
3.6-3.79	10	10	10	3	23	16	—	5	5	2	2	2	5	18	1	112
3.8-3.99	9	10	10	7	15	6	3	5	10	2	1	6	2	4	3	93
4.0-4.19	7	7	9	11	10	8	3	6	2	4	4	3	1	5	3	83
4.2-4.39	3	3	2	6	4	3	6	4	1	8	1	2	—	—	1	44
4.4-4.59	3	3	4	2	1	4	7	—	—	2	3	2	—	—	—	31
4.6-4.79	1	1	3	1	1	2	1	—	—	4	5	1	—	—	1	21
4.8-4.99	—	2	—	1	—	1	—	—	—	—	2	1	—	—	—	7
5.0-5.19	—	7	—	—	—	—	1	—	—	2	—	—	—	—	—	10
5.2-5.39	—	2	—	—	—	—	1	—	—	—	—	1	—	—	—	4
5.4-5.59	—	—	1	1	—	—	—	—	—	—	—	—	—	—	1	3
5.6-5.79	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1
5.8-5.99	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	60	66	64	55	98	98	44	30	28	28	29	30	23	67	12	732

Table IV. *Frequency distribution of solids not fat. Mixed milk.*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
7.9-7.99	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
8.0-8.09	1	—	1	—	—	1	—	—	—	—	—	—	—	—	—	3
8.1-8.19	3	1	1	—	—	—	—	—	—	—	—	—	—	—	—	5
8.2-8.29	1	—	3	—	—	2	1	—	—	—	1	—	—	5	—	13
8.3-8.39	5	1	4	1	2	5	—	—	—	—	1	—	—	7	—	26
8.4-8.49	4	2	5	2	8	15	3	1	—	—	1	2	—	16	—	59
8.5-8.59	9	1	10	9	18	25	3	2	4	—	6	4	—	12	—	103
8.6-8.69	11	—	13	14	15	13	8	7	4	2	4	5	—	11	—	107
8.7-8.79	9	2	12	10	19	17	6	5	7	5	8	7	2	4	—	113
8.8-8.89	12	13	9	7	13	6	12	7	11	5	3	2	4	5	7	116
8.9-8.99	2	12	2	7	10	9	5	7	2	8	3	2	5	4	2	80
9.0-9.09	1	11	2	3	9	3	4	—	—	4	1	2	4	1	2	47
9.1-9.19	1	13	1	1	1	1	2	1	—	2	1	4	4	1	—	33
9.2-9.29	1	9	—	1	—	—	—	—	—	—	—	2	2	1	1	17
9.3-9.39	—	1	—	—	3	—	—	—	—	2	—	—	1	—	—	7
9.4-9.49	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
9.5-9.59	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
	60	66	64	55	98	98	44	30	28	28	29	30	23	67	12	732

Table V. *Frequency distribution of protein. Mixed milk.*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
2-70-2-749	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	2
2-75-2-799	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	3
2-80-2-849	—	—	1	—	—	7	—	—	—	—	—	—	—	—	—	8
2-85-2-899	—	—	2	—	1	10	—	—	—	—	—	—	—	1	—	14
2-90-2-949	—	1	1	—	—	7	1	—	—	—	—	—	—	—	—	10
2-95-2-999	1	—	5	—	6	11	1	—	1	—	—	—	—	3	—	28
3-00-3-049	5	—	10	3	7	12	4	—	—	—	—	2	2	3	—	48
3-05-3-099	—	—	4	2	7	16	2	—	1	—	—	3	1	6	—	52
3-10-3-149	5	1	4	7	9	11	2	1	3	—	1	1	—	19	—	64
3-15-3-199	2	1	5	7	11	7	2	5	2	—	4	7	1	12	—	66
3-20-3-249	7	3	4	9	11	6	3	7	7	2	7	2	3	9	—	80
3-25-3-299	5	6	6	4	14	2	7	5	7	3	2	5	2	7	2	77
3-30-3-349	4	10	8	3	14	3	7	7	3	4	4	3	4	4	1	79
3-35-3-399	7	8	2	6	5	1	7	3	3	—	4	1	2	2	4	55
3-40-3-449	3	4	—	6	2	1	1	—	—	2	1	5	1	1	—	27
3-45-3-499	3	2	—	4	3	—	6	—	—	2	4	—	—	—	2	26
3-50-3-549	2	8	—	—	—	—	—	1	—	5	2	1	2	—	1	22
3-55-3-599	1	6	—	1	2	—	—	—	—	2	—	—	—	—	1	13
3-60-3-649	1	2	—	—	3	—	1	—	—	1	—	—	3	—	1	12
3-65-3-699	—	5	1	—	2	—	—	—	—	1	—	—	—	—	—	9
3-70-3-749	—	5	—	1	1	—	—	1	—	1	—	—	—	—	—	9
3-75-3-799	—	3	—	—	—	—	—	—	—	2	—	—	—	—	—	5
3-80-3-849	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	2
3-85-3-899	—	—	—	—	—	—	—	—	1	—	—	—	1	—	—	2
3-90-3-949	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
3-95-3-999	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
4-00-4-049	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
	46	65	64	53	98	98	44	30	28	28	29	30	23	67	12	715

From these tables the following constants have been calculated:

	Percentages of		
	Fat	Solids not fat	Protein
Standard deviation	0.5588	0.2440	0.1792
Mean	3.712	8.746	3.247
Probable error of mean	$\pm 0.014$	$\pm 0.006$	$\pm 0.004$
No. of samples	732	732	715

*Correlations.* Correlation tables showing the relationship between these three constituents have been prepared, but owing to exigencies of space these cannot be included in this paper. Graphs illustrating these tables are given herewith, with notes on each correlation.

This correlation of fat and solids not fat differs from that obtained by Tocher<sup>(1)</sup> from analyses of individual cow's milk. Tocher's correlation is linear, but in the data collected in this investigation there appears to be an indication that samples abnormally low in solids not fat are associated with a fat percentage above the average. Analysis of the data obtained reveals the fact that in the majority of herds the morning's milk was richer in solids not fat than the evening's, and the

samples from these herds may have been responsible for the parabolic type of graph. Further data are needed to determine the significance of these results.

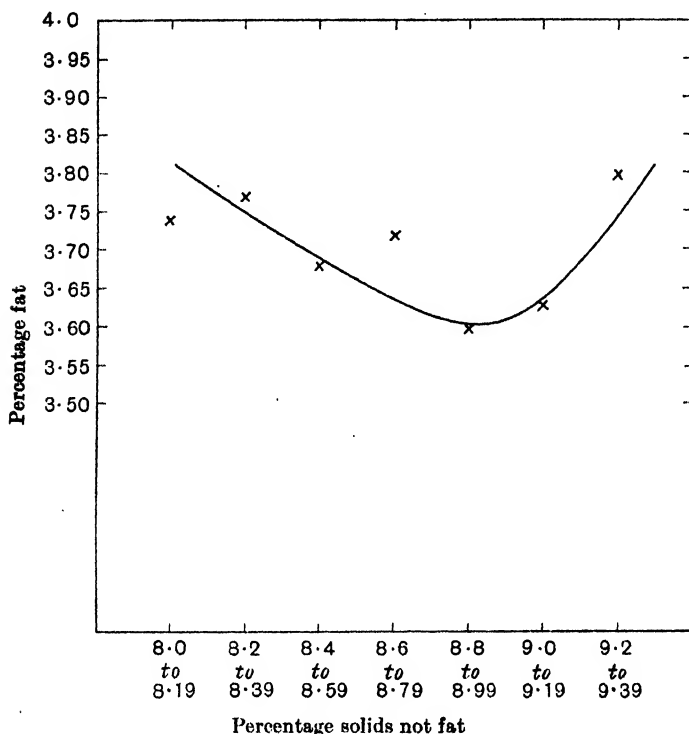


Chart I. Correlation graph of fat and solids not fat.  
Mixed milk (732 samples).

Chart II shows two graphs, one for the samples of mixed milk and the other for samples of individual cow's milk obtained during the course of this investigation from nine animals in Herd 1.

Solids not fat and protein appear to be highly correlated, and it is significant that variation in solids not fat appears to be due, partly at least, to protein, in the majority of cases. The graphs, however, suggest that in samples very low in solids not fat content, the protein percentage does not fall below a certain point, in other words the graphs are parabolic.

Tocher(1) obtained similar results for correlations of total nitrogen with solids not fat, and casein with solids not fat. Droop Richmond(2) refers to the relationship between solids not fat and protein, stating that

low solids not fat in July and August are accompanied by slightly low protein percentages. In another statement, however, he is of the opinion that any deficiency in solids not fat below 9.0 per cent. is chiefly due to

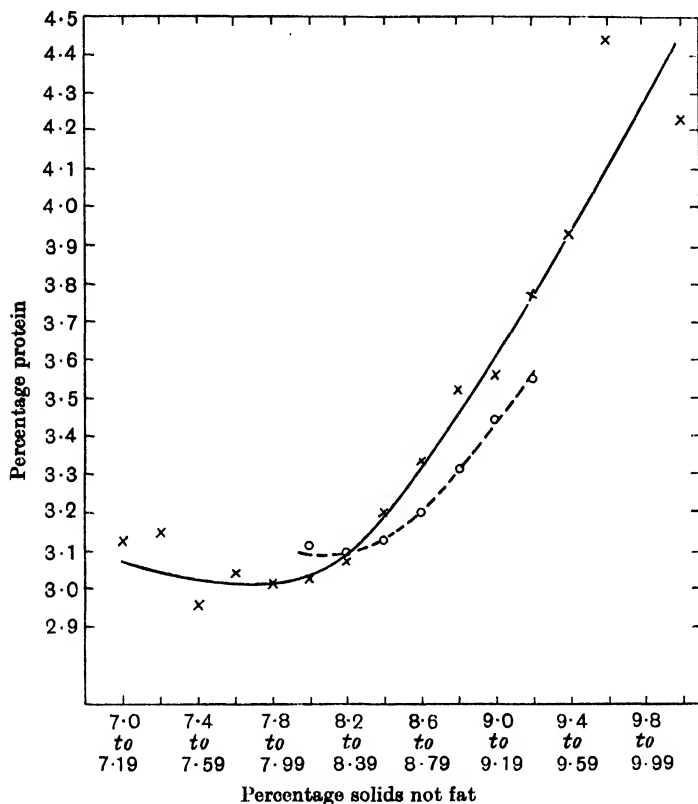


Chart II. Correlation graph of protein and solids not fat.

x — Individual milk (875 samples).    o - - - Mixed milk (715 samples).

a deficiency of milk sugar. Unfortunately it was not possible, with the limited assistance available, to determine the lactose in the samples received during this investigation, but it appears from the data obtained that protein is also responsible with lactose for deficiency in solids not fat content.

#### *Seasonal variation of protein.*

In the case of three herds complete records for one year are available. From these data an attempt has been made to correlate percentage of

protein with months of the year, but no definite relationship appears to exist. The ratio  $\frac{\text{solids not fat}}{\text{protein}}$  however shows an influence and this is indicated in Chart III. In the summer period it is apparent that the proportion of solids not fat to protein is less than in the winter months,

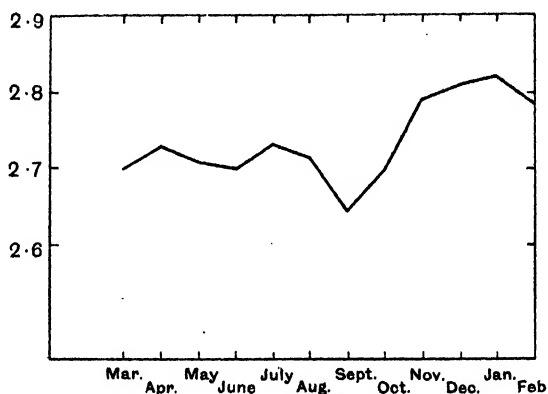


Chart III. Correlation of ratio  $\frac{\text{solids not fat}}{\text{protein}}$  with months of year.  
Herds Nos. 1, 5, 6.

and therefore one assumes that any deficiency in solids not fat in the summer is due more to lactose than protein. In winter the converse applies.

### SUMMARY.

1. Percentages of fat, solids not fat and protein were determined in over 700 samples of mixed milk from 15 herds during 1925-26. In the case of fat content, nine herds produced one or more samples below 3 per cent., one herd recording 25 per cent. of samples below this limit. With regard to solids not fat, twelve herds produced milk containing less than 8.5 per cent. on one or more occasions, the highest percentage of deficient samples recorded being 40.

2. Frequency distributions of fat, solids not fat and protein percentages in the samples analysed, are given, together with standard deviations, and mean percentages with probable errors for these three constituents.

3. Correlation tables of fat with solids not fat, and protein with solids not fat have been prepared, and graphs illustrating the variations are given.

4. With regard to the fat and solids not fat correlation, there is shown a fall in fat percentage with the solids not fat percentage to about the average (8.8 per cent.) but below this point there appears to be a rise in fat content as the solids not fat falls.

5. There is a sharp correlation between protein and solids not fat, protein content falling with the solids not fat. In the case of low percentages of solids not fat—below 8.2 per cent.—the protein fall appears to be arrested, *i.e.* the graph flattens out, and even shows a tendency to rise.

6. No definite relationship between protein percentage and season is apparent, but the ratio  $\frac{\text{solids not fat}}{\text{protein}}$  does show a correlation with the months of the year. In summer months this ratio is low but in winter it rises considerably. This appears to indicate that in summer low solids not fat percentage is due more to a deficiency in lactose than protein, but that in winter months the protein is more responsible for any solids not fat deficiency.

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- (1) TOCHER (1925). *Variations in the Composition of Milk*. H. M. Stationery Office.
- (2) DROOP RICHMOND (1920). *Dairy Chemistry*. 3rd ed. Griffin & Co.

(Received October 1st, 1926.)



## THE COMPOSITION OF MILK. PART II.

### VARIATION IN THE PERCENTAGE OF MINERAL CONSTITUENTS IN COW'S MILK, AND THEIR RELATIONSHIP WITH THE SOLIDS NOT FAT AND PROTEIN CONTENT.

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(With Ten Charts)

ALTHOUGH the mineral portion of milk amounts to less than 1 per cent. of the whole, it is a very important and complex constituent. In the milk itself there are present (a) inorganic salts, (b) inorganic bases combined with citric acid, and (c) calcium, phosphorus and sulphur combined in the amino acid groups of the caseinogen molecule.

When the solids of milk are burnt to an ash, the citrates are decomposed, leaving the bases to some extent in the form of carbonates. The calcium of the caseinogen molecule remains, but part of the phosphorus in this portion of the milk solids, with practically all the sulphur, is lost during ashing. Milk ash therefore does not wholly represent the mineral constituents of milk.

In the course of this investigation, determinations of the total ash, water soluble and insoluble ash, total calcium and total phosphorus were made, and the results obtained throw some interesting light on the variations in the composition of the mineral portion of milk.

#### ANALYTICAL METHODS.

The total ash, soluble and insoluble ash were determined as follows: 25 c.c. of the sample were weighed out in a porcelain dish, evaporated, and the residue gently heated below visible red heat, until most of the carbon had disappeared. The residue was extracted with hot water, filtered and washed. The filter paper and contents were heated in a muffle at a dull red heat until constant weight was reached. This gave the insoluble ash. To this residue was added the filtrate, and, after evaporation, the dish and contents were very gently heated until constant weight was obtained. This gave the total ash. The percentage of soluble ash was obtained by difference.

Calcium was determined in the total ash residue by the permanganate titration method.

Phosphorus in the milk was estimated by digesting 10 gm. of the sample with a mixture of nitric and sulphuric acids in a Kjeldahl flask, the phosphoric acid being determined by the ordinary molybdate-magnesia method.

At the commencement, duplicate estimations were made to test the accuracy of the methods employed. The following results were obtained:

No. of sample	Total ash	Percentages of		CaO	P <sub>2</sub> O <sub>5</sub>
		Insoluble ash	Soluble ash		
5525	0.733	0.380	0.353	0.158	0.274
„	0.738	0.382	0.356	0.158	0.272
5527	0.746	0.473	0.273	0.226	0.222
„	0.750	0.470	0.280	0.229	0.222
5533	0.708	0.504	0.204	0.132	not
„	0.714	0.504	0.210	0.136	det.

#### *Frequency distributions.*

Tables I to VII give the frequency distributions of the various mineral constituents estimated.

Constants obtained from the data in these tables are as follows:

Percentage of	Standard deviation	Mean	Probable error of mean	No. of samples
Total ash	0.0250	0.7570	± 0.0006	673
Soluble ash	0.0270	0.2663	± 0.0007	671
Insoluble ash	0.0270	0.4867	± 0.0007	671
Ratio $\frac{\text{insoluble}}{\text{soluble}}$ ash	0.2572	1.859	± 0.0066	671
Phosphoric acid	0.0183	0.2341	± 0.0005	672
Lime	0.0129	0.1843	± 0.0003	673
Ratio $\frac{\text{P}_2\text{O}_5}{\text{CaO}}$	0.1119	1.281	± 0.0029	671

Droop Richmond (1) states that 0.75 per cent. is an average figure for the ash content of milk. He also observes that the soluble ash amounts to about 0.23 per cent., the insoluble portion being 0.52 per cent. For lime (CaO) he gives 20.27 per cent. of the ash (equals 0.152 per cent. in the milk) and for phosphoric acid (P<sub>2</sub>O<sub>5</sub>) 29.33 per cent. in the ash (equals 0.220 per cent. in the milk). Armsby (2) quotes Van Slyke's average figure of 0.7 per cent for the ash in milk, and gives CaO as 20.0 per cent. and P<sub>2</sub>O<sub>5</sub> as 24.8 per cent. of the ash. Wynter Blyth (3) gives the following average figures:

CaO 22.97 per cent. and P<sub>2</sub>O<sub>5</sub> 27.03 per cent. of the ash.

It will be observed that the mean percentages of phosphoric acid and lime obtained in this research are considerably higher than those given

Table I. Frequency distribution of total ash. Mixed milk.

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0.63-0.639	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
0.64-0.649	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.65-0.659	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.66-0.669	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.67-0.679	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.68-0.689	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.69-0.699	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.70-0.709	1	1	2	1	—	—	2	—	—	—	—	—	—	—	—	2
0.71-0.719	1	5	—	1	—	2	5	1	—	—	—	1	—	—	—	10
0.72-0.729	3	11	4	6	—	6	5	1	3	—	1	4	—	2	—	18
0.73-0.739	2	10	4	12	7	9	6	4	7	1	2	4	2	4	2	51
0.74-0.749	5	11	8	13	11	18	9	5	7	—	2	4	3	5	3	77
0.75-0.759	6	13	9	6	16	18	6	4	4	1	5	4	4	25	3	124
0.76-0.769	9	7	14	5	17	12	2	8	1	1	1	3	2	15	3	111
0.77-0.779	3	3	10	5	15	14	3	2	1	7	7	2	2	8	—	91
0.78-0.789	1	—	6	—	17	7	—	—	—	—	2	2	2	8	—	81
0.79-0.799	4	—	3	2	8	8	1	1	1	2	2	2	2	1	—	39
0.80-0.809	2	2	—	—	3	2	—	—	—	4	1	—	2	—	—	34
0.81-0.819	3	2	—	—	2	2	—	—	—	1	1	1	—	—	—	16
0.82-0.829	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	12
0.83-0.839	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
0.84-0.849	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
0.85-0.859	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.86-0.869	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.87-0.879	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
42	66	60	51	97	98	44	26	24	22	24	24	24	19	68	8	673
Mean per- centages	0.701	0.743	0.754	0.740	0.762	0.756	0.729	0.748	0.740	0.787	0.761	0.748	0.753	0.744	0.740	0.7570

Percentage total ash

Table II. *Frequency distribution of insoluble ash.*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0-360-0-369	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
0-370-0-379	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-380-0-389	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-390-0-399	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-400-0-409	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
0-410-0-419	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
0-420-0-429	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	5
0-430-0-439	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19
0-440-0-449	—	—	2	3	—	1	3	—	—	—	—	—	—	—	—	24
0-450-0-459	2	2	3	2	1	3	2	—	—	—	—	—	—	—	—	51
0-460-0-469	3	2	10	2	3	10	5	1	2	—	—	1	—	—	—	74
0-470-0-479	5	1	7	13	3	12	11	3	2	—	6	3	—	—	—	96
0-480-0-489	8	7	12	12	12	21	6	2	5	—	2	4	6	8	—	112
0-490-0-499	6	8	8	8	13	16	8	7	4	2	8	3	—	3	3	63
0-500-0-509	9	8	7	1	13	13	6	6	7	3	5	3	5	1	—	38
0-510-0-519	4	6	5	3	16	7	1	4	3	4	1	4	8	—	—	18
0-520-0-529	1	12	6	3	13	10	1	1	—	4	—	4	—	2	—	9
0-530-0-539	1	6	—	2	16	1	—	1	—	1	—	1	—	—	—	5
0-540-0-549	—	7	—	1	4	3	—	—	—	2	—	—	—	—	—	1
0-550-0-559	—	3	—	1	1	1	—	—	—	1	—	—	—	—	—	2
0-560-0-569	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	1
0-570-0-579	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-580-0-589	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mean per- centages	42	66	60	51	96	98	44	26	24	22	24	24	19	67	8	671
	0-479	0-505	0-477	0-478	0-500	0-484	0-470	0-490	0-485	0-522	0-476	0-498	0-502	0-463	0-487	0-4867

Table III. *Frequency distribution of soluble ash.*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0-180-0-189	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
0-190-0-199	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	3
0-200-0-209	—	5	—	—	—	1	—	—	—	—	—	—	—	—	—	8
0-210-0-219	—	1	—	—	1	—	1	1	—	—	—	—	—	—	—	8
0-220-0-229	—	17	2	1	4	2	1	1	—	1	—	2	—	—	—	32
0-230-0-239	—	14	4	1	8	7	3	1	2	3	—	3	6	—	1	52
0-240-0-249	—	8	5	5	7	11	5	3	7	5	—	3	1	4	2	70
0-250-0-259	2	8	5	13	24	7	12	8	5	2	2	6	3	8	1	108
0-260-0-269	8	4	7	18	18	18	10	5	6	2	2	3	3	9	3	116
0-270-0-279	5	2	6	10	13	11	8	4	4	5	5	—	—	—	—	82
0-280-0-289	6	2	5	—	15	15	3	3	—	—	2	1	3	7	—	62
0-290-0-299	7	1	12	1	5	15	1	—	—	1	1	2	—	13	—	58
0-300-0-309	4	—	8	1	—	7	1	—	—	1	5	1	1	9	—	38
0-310-0-319	2	—	2	—	—	2	—	—	—	1	—	—	—	6	—	13
0-320-0-329	2	1	—	—	—	—	—	—	—	—	—	—	—	1	1	6
0-330-0-339	2	—	3	1	1	2	—	—	—	1	1	—	—	—	—	11
0-340-0-349	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1
0-350-0-359	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-360-0-369	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-370-0-379	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mean per- centages	42	66	60	51	96	98	44	26	24	22	24	24	19	67	8	671
	0-282	0-238	0-277	0-262	0-262	0-272	0-259	0-258	0-255	0-265	0-285	0-250	0-251	0-281	0-253	0-2663

Table IV. *Frequency distribution of ratio  $\frac{\text{insoluble}}{\text{soluble}}$  ash.*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1-00-1-09	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
1-10-1-19	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
1-20-1-29	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	1
1-30-1-39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
1-40-1-49	1	—	3	—	1	1	—	—	—	—	1	—	—	—	—	8
1-50-1-59	6	—	5	2	—	4	—	—	—	—	—	—	—	—	—	35
1-60-1-69	7	2	11	—	1	13	2	—	—	1	2	1	—	15	—	55
1-70-1-79	9	3	10	3	10	14	4	2	2	1	2	3	2	9	—	69
1-80-1-89	7	6	4	14	15	20	15	5	6	2	2	4	2	6	3	120
1-90-1-99	3	8	5	6	29	10	13	7	3	3	5	4	4	8	2	107
2-00-2-09	1	7	3	2	11	10	5	1	7	4	2	2	2	3	1	102
2-10-2-19	1	10	3	3	7	6	4	1	6	2	2	3	5	1	1	62
2-20-2-29	—	12	—	—	5	3	—	2	—	5	—	3	1	—	—	43
2-30-2-39	—	5	1	1	5	1	—	—	—	3	—	—	1	—	—	26
2-40-2-49	1	3	—	—	—	1	—	1	—	—	—	1	1	—	—	17
2-50-2-59	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	8
2-60-2-69	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	4
2-70-2-79	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	2
2-80-2-89	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2
	42	66	60	51	96	98	44	26	24	22	24	24	19	68	7	671
Mean per- centages	1-77	2-14	1-75	1-78	1-93	1-80	1-81	1-91	1-89	1-97	1-69	2-01	2-02	1-66	1-93	1-859

Ratio

## The Composition of Milk

Table V. Frequency distribution of phosphoric acid.

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0-175-0-1799	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
0-180-0-1849	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	1
0-185-0-1899	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-190-0-1949	—	—	—	—	—	1	—	1	—	—	1	—	—	—	—	6
0-195-0-1999	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1
0-200-0-2049	1	—	—	—	—	3	2	1	—	—	—	—	—	—	—	14
0-205-0-2099	4	1	3	—	3	4	2	—	—	—	1	—	—	4	—	27
0-210-0-2149	4	1	1	6	3	11	1	3	1	—	1	2	—	5	1	42
0-215-0-2199	4	1	1	5	7	16	5	2	4	—	3	1	—	8	1	67
0-220-0-2249	12	2	3	5	12	14	2	6	1	—	2	—	—	11	2	74
0-225-0-2299	3	3	7	2	11	17	—	1	4	1	4	3	1	11	2	70
0-230-0-2349	3	1	4	4	13	5	4	3	1	—	3	3	2	4	1	53
0-235-0-2399	3	8	5	5	15	4	5	2	3	4	3	5	4	4	—	65
0-240-0-2449	3	6	9	6	9	8	4	2	3	2	2	2	4	3	—	66
0-245-0-2499	2	7	4	3	9	7	9	—	4	3	—	5	—	2	—	52
0-250-0-2549	2	13	11	3	6	5	1	1	1	3	3	2	—	—	—	51
0-255-0-2599	1	12	2	1	4	1	1	1	1	2	—	1	3	—	—	30
0-260-0-2649	—	7	1	—	3	1	2	1	—	4	1	1	1	—	—	21
0-265-0-2699	—	3	1	1	1	1	1	—	—	1	—	3	—	—	—	14
0-270-0-2749	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	2
0-275-0-2799	—	1	2	—	2	—	3	—	—	1	—	1	—	—	—	8
0-280-0-2849	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	3
0-285-0-2899	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-290-0-2949	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1
0-295-0-2999	—	—	—	—	—	—	—	1	—	1	—	—	—	—	—	2
0-300-0-3049	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0-305-0-3099	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
42	66	60	51	98	98	98	44	26	23	22	24	24	19	68	7	672
Mean per- centages	0-231	0-247	0-238	0-229	0-235	0-227	0-238	0-232	0-234	0-253	0-230	0-243	0-250	0-220	0-222	0-2341

Percentage P<sub>2</sub>O<sub>5</sub>

Table VI. *Frequency distribution of lime.*

Herd	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
0-135-0-1399	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
0-140-0-1449	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
0-145-0-1499	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	2
0-150-0-1549	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	9
0-155-0-1599	—	—	2	—	—	1	1	—	—	—	—	—	—	5	—	22
0-160-0-1649	—	—	5	—	1	3	2	—	—	—	—	—	—	11	—	37
0-165-0-1699	—	2	5	4	7	3	3	3	1	—	2	—	—	10	—	81
0-170-0-1749	6	6	16	5	10	10	7	2	2	—	6	—	1	13	1	109
0-175-0-1799	8	6	8	7	8	17	9	4	10	5	3	7	—	6	4	—
0-180-0-1849	7	11	10	11	13	9	5	4	2	3	3	5	3	6	3	95
0-185-0-1899	7	9	8	7	20	18	4	5	5	5	6	4	10	4	—	112
0-190-0-1949	7	9	6	7	12	13	3	3	—	2	2	2	2	4	—	72
0-195-0-1999	2	10	3	6	11	13	6	—	1	2	—	2	1	—	—	57
0-200-0-2049	3	3	—	1	6	5	2	1	1	2	2	2	2	1	—	29
0-205-0-2099	2	3	—	2	4	2	1	2	—	1	2	1	—	—	—	21
0-210-0-2149	—	4	—	—	1	4	1	—	—	1	—	—	—	—	—	11
0-215-0-2199	—	2	—	—	5	—	—	1	1	—	—	1	—	—	—	8
0-220-0-2249	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
0-225-0-2299	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Mean per- centages	42	66	60	51	98	98	44	25	24	22	24	24	19	68	8	673
	0-185	0-190	0-178	0-183	0-188	0-186	0-182	0-185	0-184	0-192	0-183	0-188	0-188	0-171	0-178	0-1843

Percentage CaO



## The Composition of Milk

Table VII. Frequency distribution of ratio  $\frac{\text{phosphoric acid}}{\text{lime}}$ .

Ratio	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Herd																
0.95-0.999	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1
1.00-1.049	—	—	—	—	—	3	1	—	—	—	—	—	—	—	—	6
1.05-1.099	3	2	2	2	4	3	2	1	1	1	—	1	—	1	—	21
1.10-1.149	4	—	—	—	7	10	5	2	—	—	2	—	—	—	—	37
1.15-1.199	9	6	1	16	13	25	1	5	1	—	4	—	—	3	—	89
1.20-1.249	10	6	4	10	25	16	7	5	3	2	4	2	—	4	1	112
1.25-1.299	10	18	11	5	21	13	6	5	9	1	7	3	2	14	1	136
1.30-1.349	4	14	15	5	11	17	6	1	5	4	3	6	4	14	1	110
1.35-1.399	1	7	10	9	8	8	4	2	1	8	2	4	4	9	—	77
1.40-1.449	1	4	3	1	3	2	2	1	1	3	—	1	—	4	—	39
1.45-1.499	—	—	2	—	4	—	6	—	—	—	2	2	1	4	—	23
1.50-1.549	—	1	1	—	2	—	3	1	—	—	—	—	—	1	—	10
1.55-1.599	—	—	1	—	—	—	1	1	—	—	—	—	—	—	—	3
1.60-1.649	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	2
1.65-1.699	—	—	1	—	—	—	—	—	—	—	—	—	1	—	—	2
1.70-1.749	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
1.75-1.799	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1
1.80-1.849	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.85-1.899	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
42	66	60	51	98	98	98	44	25	23	22	24	24	19	68	7	671
Mean per- centages	1.22	1.37	1.34	1.25	1.26	1.22	1.30	1.26	1.27	1.33	1.27	1.30	1.33	1.29	1.25	1.281

by the above authorities. This may be due to the analytical methods employed, particularly as regards the  $P_2O_5$  figures.

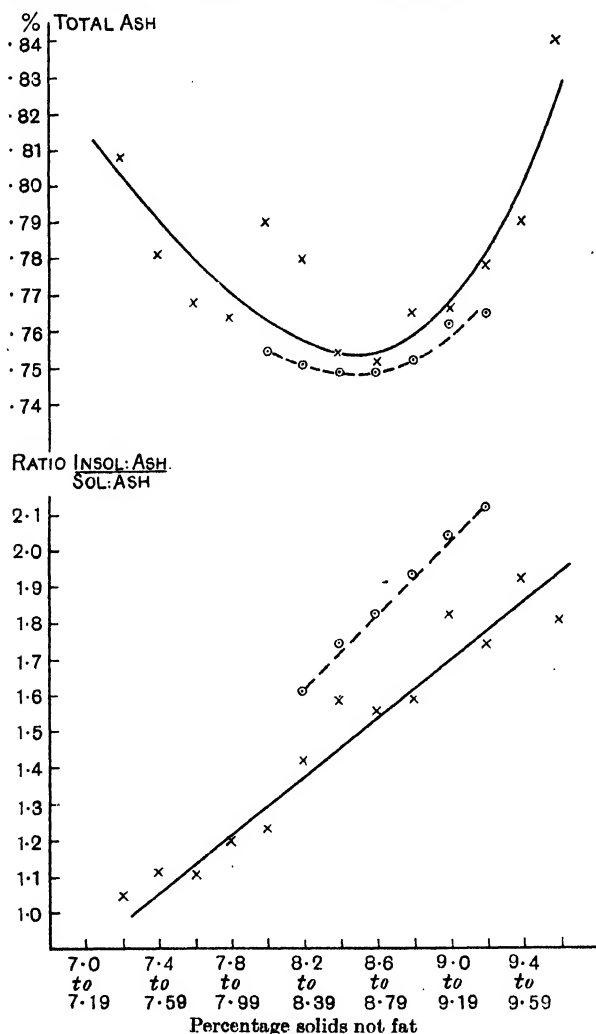
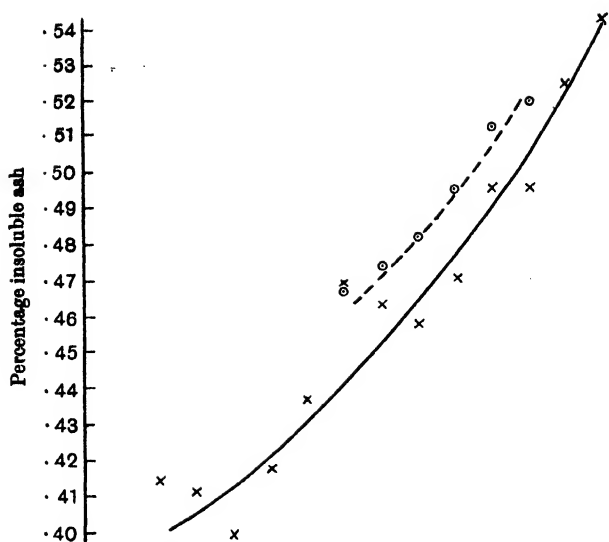


Chart I. Correlation of solids not fat with total ash, and ratio  $\frac{\text{insol. ash}}{\text{sol. ash}}$ .

x — Individual cow's milk (438 samples).      o - - - Mixed milk (672 samples).

This constituent, formerly, was determined in the ash, but nowadays it is determined directly in the milk owing to loss of phosphoric acid during ashing.



Correlation of solids not fat with insoluble ash.

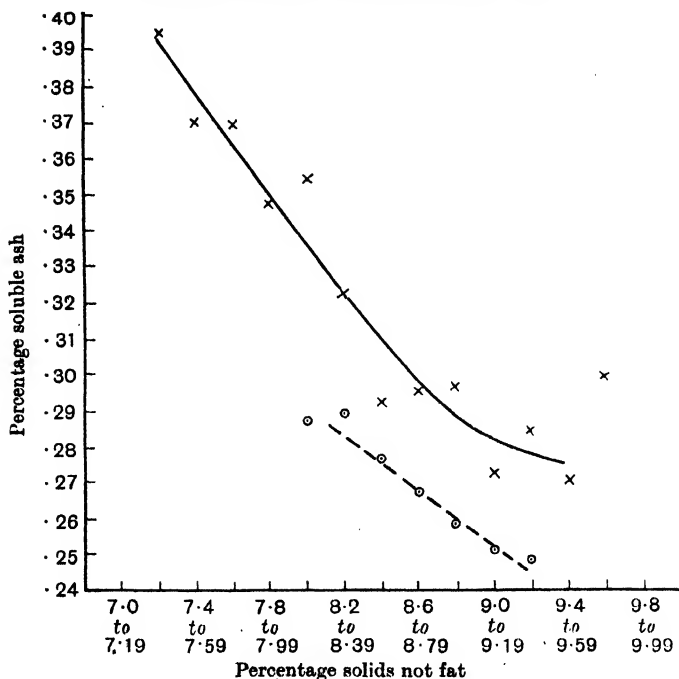


Chart II. Correlation of solids not fat with soluble ash.

x — Individual cow's milk (438 samples).      o - - - Mixed milk (672 samples).

With regard to the ratio  $\frac{P_2O_5}{CaO}$  reference is made to this figure by Ruston(4) in a paper on air pollution by coal smoke. He observes that this ratio in normal milk rarely falls below 1.25, but that in abnormal

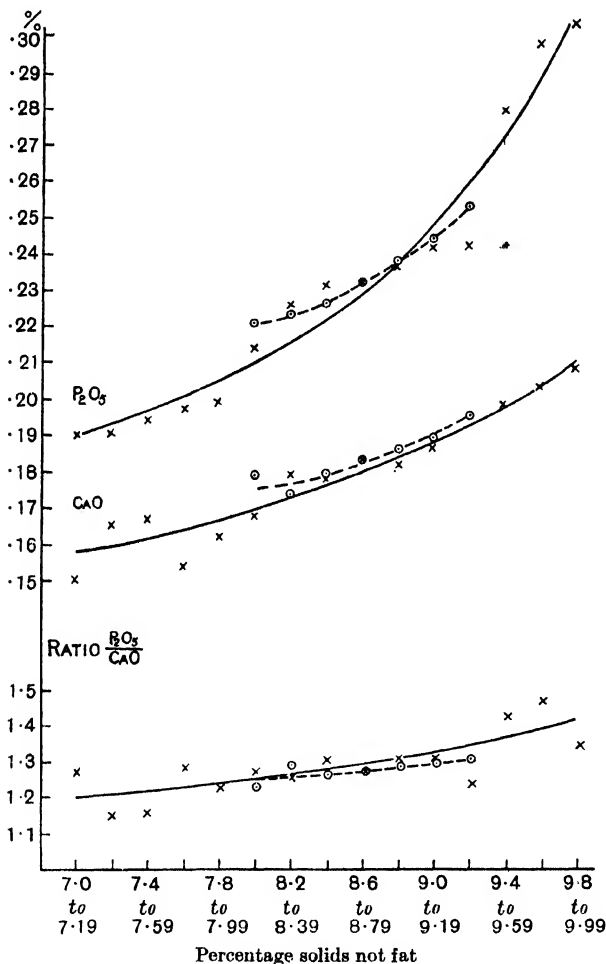


Chart. III. Correlation of solids not fat with

(1)  $P_2O_5$ . (2)  $CaO$ . (3) Ratio  $\frac{P_2O_5}{CaO}$ .

x — Individual cow's milk (412 samples).      o — Mixed milk (672 samples).

milks from smoke polluted areas he found much higher ratios, rising in some samples to nearly 2.

## The Composition of Milk

*Correlations.* Various correlations between the mineral constituents and solids not fat and protein have been prepared, but cannot be included owing to lack of available space. Graphs illustrating these correlations are shown in Charts I to V. For purposes of comparison

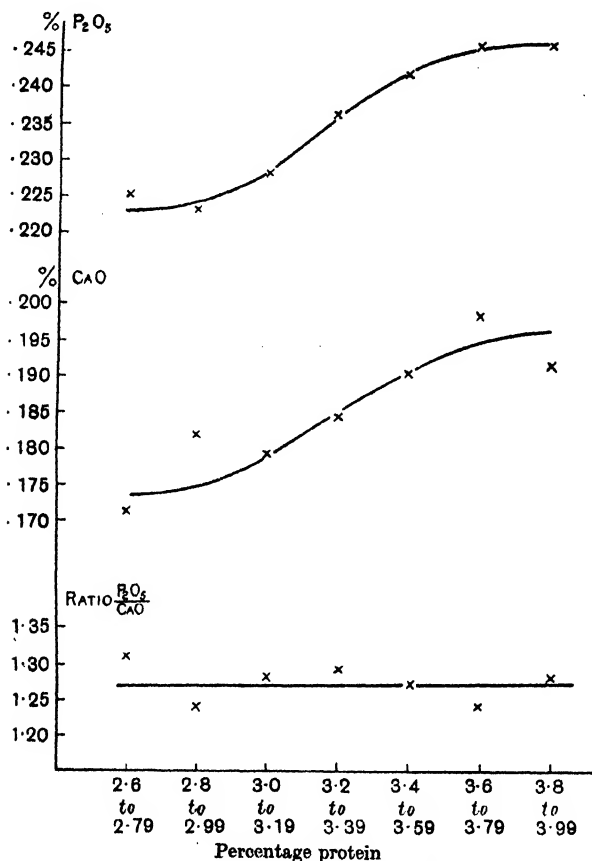


Chart IV. Correlation of protein with

(1)  $P_2O_5$ . (2)  $CaO$ . (3) Ratio  $\frac{P_2O_5}{CaO}$ .

Mixed milk.

curves are included in some charts representing the analytical data obtained from over 400 samples of individual cow's milk, taken from 15 cows in Herd 1 at various periods during 1923-5.

## OBSERVATIONS AND DISCUSSION.

*Total ash.* The ash percentage of milk varies to some extent, extreme limits in the samples of mixed milk being 0.63 per cent. and 0.87 per cent.

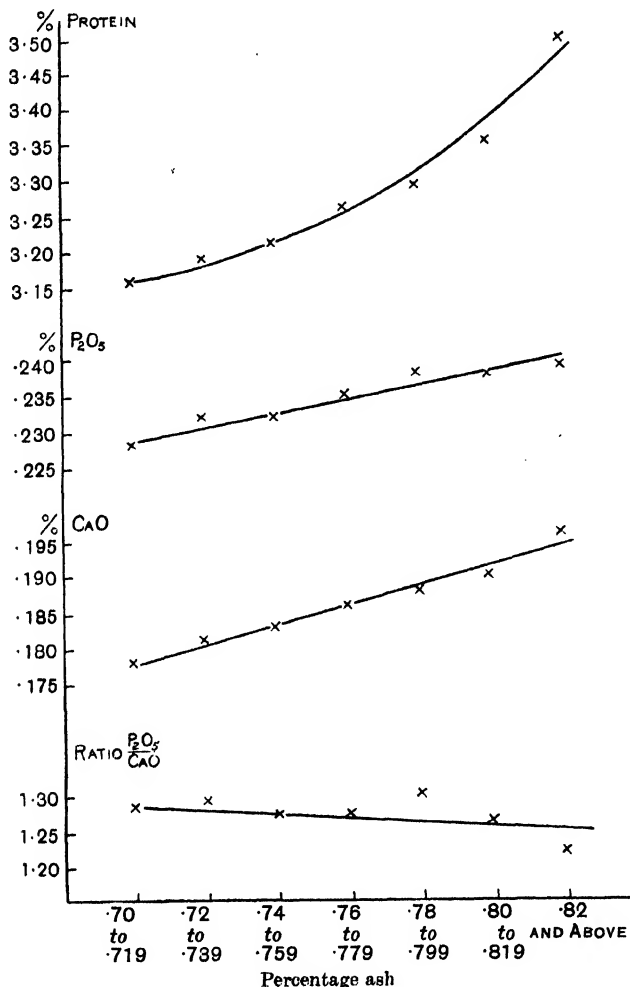


Chart V. Correlation of ash with  
 (1) Protein. (2)  $P_2O_5$ . (3) CaO. (4) Ratio  $\frac{P_2O_5}{CaO}$ .  
 Mixed milk.

Correlated with solids not fat, the ash percentage falls with this constituent until about 8.7 per cent. solids not fat is reached when the curve

flattens out and a rise in ash occurs as the solids not fat percentage falls still further. In Tocher's memoir(5) is given a curve illustrating this correlation and this curve is also parabolic, showing a flattening out in the region of 8.6 per cent. solids not fat, but, since the solids not fat scale does not fall below 8.4 per cent., the apparent rise in ash percentage

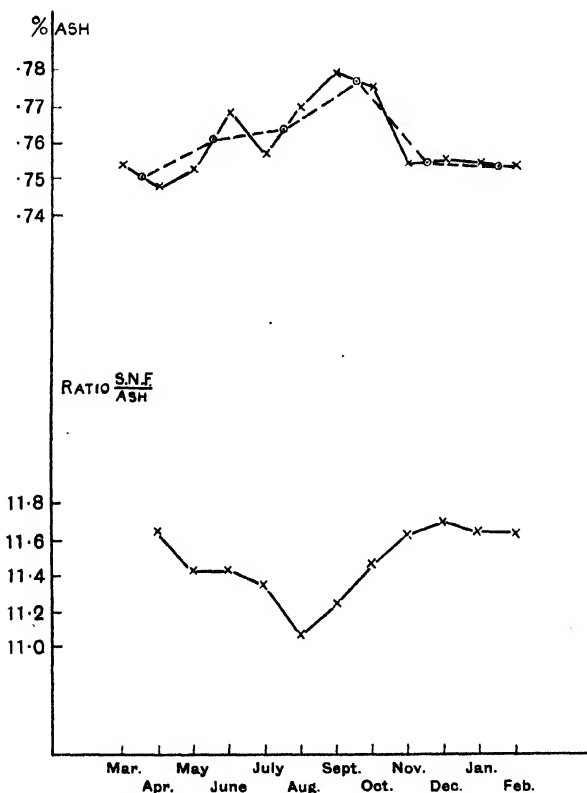


Chart VI. Correlation of total ash and ratio  $\frac{\text{S.N.F.}}{\text{ash}}$  with months of the year.

Average for mixed milk of 3 herds during one year.

x — Monthly averages.

o - - Bi-monthly averages.

for low solids not fat values mentioned above, cannot be confirmed or otherwise from Tocher's results. There appears to be a correlation between ash and protein, due undoubtedly to the calcium and phosphorus contained in the caseinogen.

The ash content of milk appears to have a seasonal variation. Table VIII shows the frequency distribution of the ash in samples from three

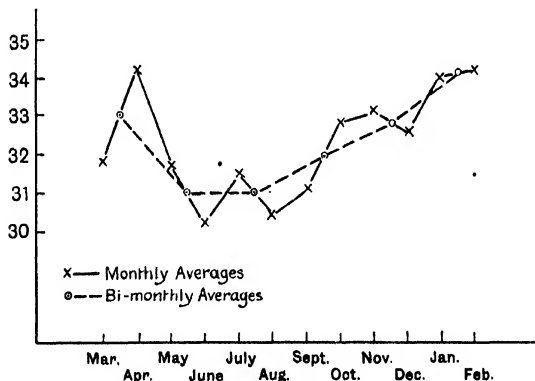
Table VIII. *Correlation of ash with months of year.*

Ash %	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
0.700-0.719	1	—	—	—	—	—	—	—	1	—	1	4
0.720-0.739	6	8	3	1	2	—	—	1	1	4	4	35
0.740-0.759	4	10	11	8	8	9	3	4	8	5	5	85
0.760-0.779	8	5	8	11	7	6	9	8	7	3	8	88
0.780-0.799	3	2	4	7	1	3	9	7	6	5	4	52
0.800-0.819	1	—	—	1	1	3	3	4	—	—	1	16
0.820-0.839	—	—	—	—	—	1	1	—	—	—	—	2
0.840-0.859	—	—	—	—	—	—	1	—	—	—	—	1
	23	25	26	28	19	22	26	24	23	17	23	283



Correlation of soluble ash with months of the year.

Mixed milk of herds 1, 5, 6.

Chart VII. Correlation of ratio  $\frac{\text{S.N.F.}}{\text{sol. ash}}$  with months of the year.

Mixed milk of herds 1, 5, 6.

herds, for each month of the year. Figures from the other herds cannot be included, since complete year's records were not available in these cases. This seasonal variation is graphically illustrated in Chart VI



where it will be seen that the ash percentage reaches a maximum in the summer (the grass months) and a minimum in the winter. It is significant that the ash content should reach its highest point when the solids not fat percentage is low.

The ratio  $\frac{\text{solids not fat}}{\text{ash}}$  correlation with months of the year is also shown in Chart VI.

*Soluble and insoluble ash.* The soluble portion of milk ash consists chiefly of sodium chloride whilst calcium phosphate is the main constituent of the insoluble ash. It is found that the percentages of soluble and insoluble ash and the ratio of the two are highly correlated with the percentages of solids not fat.

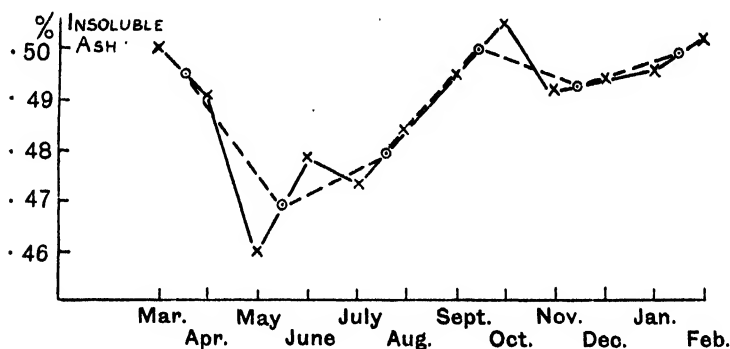


Chart VIII. Correlation of insoluble ash with months of the year.

Mixed milk of herds 1, 5, 6.

x — Monthly averages.

o - - - Bi-monthly averages.

It is apparent from the graphs in Chart II that milk low in solids not fat is rich in soluble ash and poor in insoluble ash. This point may have considerable physiological importance. Porcher(8) states that sodium chloride (the principal salt in the soluble ash) is one of the most variable constituents of milk, and the amount present in milk is influenced by reason of its function in maintaining osmotic equilibrium in milk secretion. Quoting analytical data supplied by Böggild, Porcher finds that a high chloride content in milk is associated with a low percentage of lactose, and *vice versa*. This statement, when considered in conjunction with the solids not fat and soluble ash correlation, leads one to infer that deficiency in solids not fat is due in some measure to low lactose content.

Soluble ash shows considerable variation with months of year. In Chart VII it will be seen that the percentage of soluble ash is considerably higher in the summer than in the winter. This is the reverse of the solids not fat monthly variation. The correlation of the ratio  $\frac{\text{solids not fat}}{\text{soluble ash}}$  with months of the year is illustrated by a graph in Chart VII. Assuming Porcher's conclusions regarding the relation of lactose to sodium chloride to be correct, high soluble ash in milk indicates low lactose content, and therefore low percentages of solids not fat in the summer months are due to a deficiency in lactose. This assumption is further strengthened by the correlation of lime and solids not fat, referred to later in this paper (see p. 91).

The insoluble ash percentage falls with the solids not fat but the rate of fall is not of the same magnitude as the rate of rise in the case of the soluble ash. The graph, moreover, is parabolic and is comparable with the protein—solids not fat graph (see Chart II in Part I).

Since a portion of the lime and phosphoric acid in the insoluble ash is derived from the caseinogen in the milk, the similarity in these curves is obvious.

*Lime and phosphoric acid.* The frequency tables nos. V and VI indicate that the percentage of phosphoric acid is much more variable than the percentage of lime. Correlated with solids not fat, there is shown a decided fall with the percentage of solids not fat, except at low values where the curves of both of these constituents appear to flatten out (see Chart III), and this is most noticeable in the case of lime. The phosphoric acid curve, however, is somewhat steeper than the lime curve, and this is indicated in the graph showing the correlation of solids not fat with the ratio  $\frac{P_2O_5}{CaO}$ , a slight fall being shown. This fall, however, is small, and it therefore appears that milk low in solids not fat is not necessarily associated with an abnormal  $\frac{P_2O_5}{CaO}$  ratio. It has been observed that this ratio is very variable in individual samples. Extremes in the case of mixed milks were 0.97 and 1.87, and one figure as low as 0.89 was obtained in the case of a sample of milk from an individual cow. It will be observed that at the 8.6 to 8.79 solids not fat point of the curves, the figures obtained for lime and phosphoric acid respectively are identical for the mixed milk and the individual cow's milk, viz.: 0.183 per cent. CaO and 0.232 per cent.  $P_2O_5$ , the ratio being 1.27. These figures approximate to the means for these determinations.

## The Composition of Milk

With regard to seasonal variation, phosphoric acid appears to be influenced but little, with the exception of a slight rise in May and June and a fall in July. Lime, on the other hand, shows a steady fall in the late spring and summer, reaching a low percentage in August, following which a sharp rise occurs. The graph illustrating this correlation (Chart IX) is comparable with the observed seasonal variation of solids not fat.

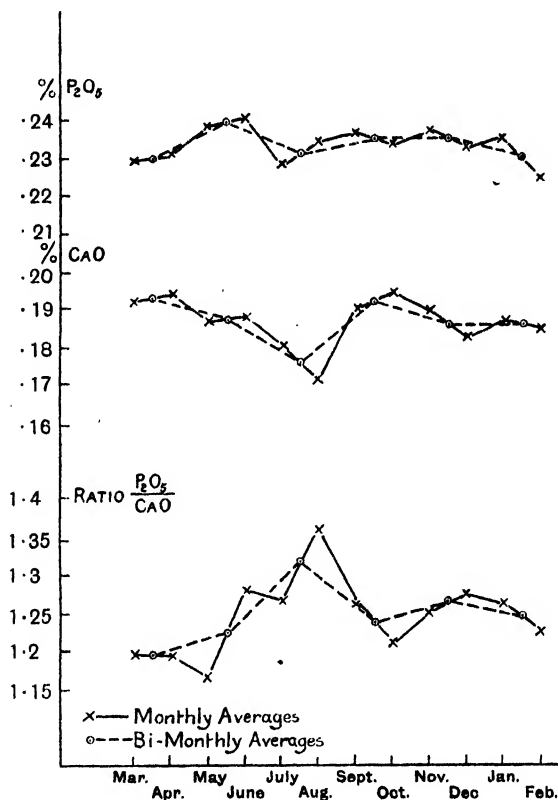


Chart IX. Correlation of  $P_2O_5$ ,  $CaO$  and Ratio  $\frac{P_2O_5}{CaO}$  with months of year.

Mixed milk of herds 1, 5, 6.

The magnitude of the fall in the  $CaO$  graph, however, appears to be greater, and, as a result, the correlation graph of the ratio  $\frac{\text{solids not fat}}{CaO}$  (Chart X) with months of the year shows a considerable rise in August.

It therefore appears that in the summer period when the solids not fat percentage is low, the lime percentage is also low. This is not due to protein deficiency since the ratio  $\frac{\text{solids not fat}}{\text{protein}}$  is low at that period (see Chart III, Part I), and any deficiency in solids not fat in the summer months must therefore be due in the main to lactose. It is suggested that this low lime content in the summer months is due to a deficiency of calcium compounds other than the combined calcium in the caseinogen, but this can only be determined with any degree of certainty by more

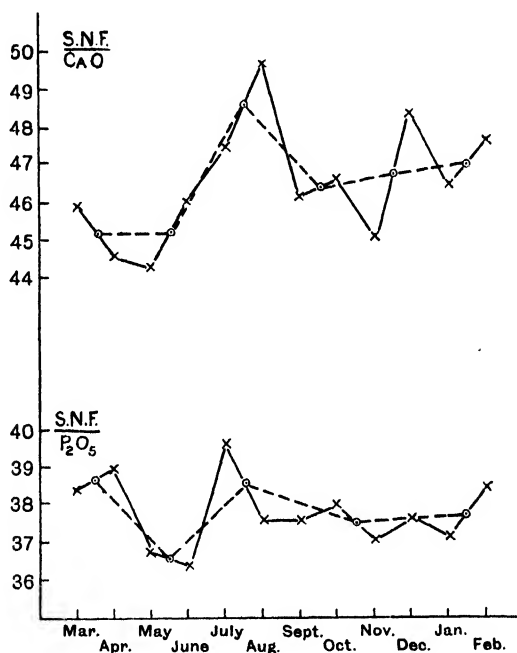


Chart X. Correlation of  $\frac{\text{S.N.F.}}{\text{CaO}}$ ,  $\frac{\text{S.N.F.}}{\text{P}_2\text{O}_5}$ , with months of year.

Mixed milk of herds 1, 5, 6.

x — Monthly averages.

o — Bi-monthly averages.

detailed analyses than were possible in this investigation. An interesting physiological point is thereby raised. Does the balance of the mineral constituents of milk alter during the year, and, if so, is this change due to the great differences in the nature of winter compared with summer feeding? In other words, does grass influence the composition of the mineral constituents of milk?

The graph for the correlation of the ratio  $\frac{\text{solids not fat}}{\text{P}_2\text{O}_5}$  with months of the year is somewhat similar to the corresponding lime curve, but the rise occurs a month earlier. The ratio  $\frac{\text{P}_2\text{O}_5}{\text{CaO}}$  shows a definite variation with months of the year (see Chart IX), the graph being practically the reverse of the lime graph.

#### SUMMARY.

1. 670 samples of the mixed milk from 15 herds were analysed, and the average percentages of total ash, soluble ash, insoluble ash, lime and phosphoric acid are given.

2. Tables showing frequency distributions are also given, with the standard deviation, mean and probable error of mean for each constituent determined.

3. Various correlations of these constituents with solids not fat and protein have been prepared, and these correlations are illustrated by graphs.

It is observed that the total ash falls with the solids not fat until low values of solids not fat are reached, when the ash content appears to rise. This variation is confirmed by a curve illustrating the variation in ash content of samples of individual cow's milk. Soluble ash rises as the solids not fat falls, but the insoluble ash shows a reverse variation. Lime and phosphoric acid both fall with the solids not fat.

4. Complete year's records of the analysis of the milk of three herds are available and correlations of the various constituents enumerated above, with months of the year, are graphically shown. It appears that the total ash and soluble ash reach a maximum in summer but give low average figures in the winter months, whilst the insoluble ash falls in spring and rises in the autumn. Phosphoric acid shows little change during the year, but the lime content falls in spring and summer, rising again in the autumn.

The authors wish to express their thanks to Mr G. O. Fox, Assistant Analyst at the Midland College, who undertook a portion of the analytical work at the commencement of the investigation, and also to Mr H. G. Sanders of the School of Agriculture, Cambridge, for helpful advice in connection with the statistical treatment of the data. Their thanks are also due to the various owners of the herds and their staffs for giving facilities for, and help in, the taking of samples.

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# LOSSES OF ADDED PHOSPHATE BY LEACHING FROM N. WELSH SOILS.

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THE phosphorus status of soils is one of the most important factors in permanent and successful agriculture. Nitrogen can be obtained from the atmosphere either by the agency of bacteria or by industrial synthetic processes. Potash is a relatively abundant constituent of most of the rocks from which soils originate. The supplies of phosphorus are, however, limited, for the ordinary soil-forming rocks do not contain more than a fractional percentage of this element. For the maintenance of crop production at a high or even moderate level, external supplies of phosphatic fertilisers appear to be a necessity. The world's resources of phosphorus are by no means unlimited and the economical use of this element is urged both by immediate circumstances and by a reasonable regard to future developments.

It has been generally assumed that the losses of phosphoric acid by leaching are insignificant. In the classical investigations at Rothamsted, the annual losses of phosphoric acid do not amount to more than 2 lb. per acre. In the more recent lysimeter experiments at Craibstone, Aberdeen, Hendrick<sup>(1)</sup> found the losses of phosphoric acid to be of the order of 1 lb. per acre. Dyer<sup>(2)</sup> concluded from the Rothamsted Broadbalk Field results that added phosphorus was generally retained in the top layer of the soil, except in the case of farmyard manure plots, where there was evidence of some descent. Blair and Prince<sup>(3)</sup> in New Jersey found a small loss of phosphorus by leaching. Harrison and Das<sup>(4)</sup> found evidence for the downward movement of phosphorus in non-calcareous soils, although in calcareous soils there was an accumulation in the top layers.

The annual removal of phosphorus in crops is generally not more than about 30 lb. per acre. The net loss with ordinary British arable cultivation is considerably less than this, yet in order to maintain production at a satisfactory level it would seem to be necessary to supply phosphorus in the form of fertilisers at a rate greater than the loss by crops.

The beneficial effect of basic slag on poor grassland is a matter of

common knowledge. It has appeared from numerous results, particularly in N. Wales, that the big initial improvement produced by slag is not maintained and that there is a tendency for improved pastures to revert to their original state unless further dressings of slag are applied. In N. Wales it has been found that the effect of slag on certain rough grazings practically vanishes after about six to eight years. Assuming that this is due to a shrinkage in the supply of phosphoric acid available for the grass herbage, we may explain it in one or more of the following ways.

(1) Losses of phosphorus due to grazing animals. This could not account for the known disappearance of the effect of 200 lb.  $P_2O_5$  in six years in certain of our Welsh experiments. (2) Reversion of added phosphates to insoluble forms. If this were the case the added phosphates would still be found in the top layers. This possibility has been examined and it will be seen from the results obtained that no such retention takes place. (3) The phosphorus may be leached from the surface layers of the soil by percolating water. In this connection the heavy leaching consequent on the humid conditions of N. Wales must be remembered.

Amongst the most promising tendencies in pedology is the study of soils in profile, a method which in the hands of Russian workers has yielded valuable information on the origin and metamorphoses of soils. It was felt by us that the application of these methods to the soil phosphorus problem might yield profitable results. Fortunately there was available for the work a good selection of phosphatic experiments on poor grass-land and these were made the material of our studies. In the main series of experiments we have confined our attention to three sets of plots, namely (1) those which had received 200 lb.  $P_2O_5$  in the form of high grade slag in 1914, (2) those which had received 200 lb.  $P_2O_5$  in 1914 and a similar dressing in 1922, and (3) the control plots. These comparisons were available at two centres. At two other centres the 1922 dressing was not applied and the experiments related only to 1914 slag and control, whilst the plots at the Aber centre belong to a different series of experiments. At each centre and from each plot composite samples were taken to represent successive 3" layers down to 18". Owing to the stony nature of our Welsh soils it was always necessary to carry out the sampling with a spade. This was effected by digging out rectangular holes to the requisite depth and sampling from the sides in successive 3" layers.

The following determinations were made on each individual profile sample.

(1) Mechanical analysis. The determinations were made before the



*Losses of Added Phosphate by Leaching*Table I. *Analytical Data for Control Plots.*

	Penlan, Llangollen						Ffyddion, Caerwys					
	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)
Fine gravel	1.9	3.6	3.3	3.3	5.8	15.9	0.4	1.4	2.0	1.5	1.8	3.2
Coarse sand	0.6	0.9	0.7	0.7	1.0	8.3	6.0	7.7	8.4	8.8	8.0	10.7
Fine sand	7.6	9.4	9.6	13.0	14.3	15.1	14.7	14.5	14.1	14.3	14.9	15.3
Silt ...	18.7	22.8	27.0	27.3	27.5	21.3	14.5	14.8	15.3	16.5	17.3	19.8
Fine silt ...	18.5	23.5	25.0	24.9	22.9	21.1	23.5	24.5	24.8	25.3	25.2	23.5
Clay ...	21.0	22.3	20.3	17.7	15.7	9.9	18.9	19.5	21.5	20.5	20.8	20.3
Hyg. moisture	4.4	3.0	3.4	2.6	2.5	2.5	4.1	3.2	2.3	2.4	1.8	1.2
Ignition ...	21.0	12.8	10.5	8.3	7.7	6.9	14.2	10.6	9.0	8.4	7.2	2.9
R <sub>2</sub> O <sub>3</sub> ...	11.7	12.3	13.0	13.3	13.1	14.3	12.7	12.0	12.7	12.5	13.2	12.3
SiO <sub>2</sub> ...	8.2	6.6	5.5	5.7	6.3	6.2	6.5	6.0	6.2	6.3	6.5	6.3
Exchangeable CaO	0.186	0.176	0.188	3.145	0.107	0.070	0.409	0.386	0.404	0.395	0.289	0.221

	Maesmynan, Caerwys						Dolwgan, Garn Dolbenmaen					
	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)
Fine gravel	8.7	14.0	23.8	23.9	20.7	24.3	2.4	6.2	7.3	7.6	9.6	9.8
Coarse sand	21.6	19.3	16.4	17.0	18.4	18.5	2.0	2.8	2.9	2.2	2.0	4.8
Fine sand	13.6	14.1	12.9	13.0	14.9	13.6	4.8	10.3	9.1	9.9	9.4	8.6
Silt ...	6.0	6.8	8.7	10.0	11.0	10.5	6.8	10.8	8.8	8.5	11.5	7.3
Fine silt ...	10.5	14.0	14.5	14.7	14.5	13.0	20.3	23.5	24.3	31.0	23.5	23.0
Clay ...	9.0	9.8	10.3	9.3	9.3	9.8	29.5	27.0	28.4	27.5	32.5	30.3
Hyg. moisture	3.9	3.2	2.2	2.0	2.0	2.0	7.5	3.2	3.3	3.1	3.2	3.4
Ignition ...	17.2	12.2	9.1	7.4	7.1	6.3	25.7	13.9	12.3	9.6	9.7	10.2
R <sub>2</sub> O <sub>3</sub> ...	9.4	10.8	11.6	11.5	10.6	10.3	17.7	17.7	18.8	17.6	17.6	18.9
SiO <sub>2</sub> ...	5.4	5.5	5.1	5.2	5.1	4.6	7.7	5.2	5.5	5.4	4.7	4.7
Exchangeable CaO	0.151	0.150	0.114	0.117	0.148	0.099	0.060	0.042	0.037	0.037	0.038	0.030

	Ffridd, Aber (A)						Ffridd, Aber (B)					
	(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)
Fine gravel	10.6	21.3	26.4	30.7	34.7	36.2	10.3	16.2	28.6	26.7	25.7	30.2
Coarse sand	5.3	6.4	6.4	8.3	9.8	8.1	9.2	8.5	8.3	9.0	10.7	9.5
Fine sand	14.4	6.1	8.2	8.0	7.9	7.3	7.4	8.9	9.1	9.4	8.6	9.9
Silt ...	9.5	9.8	10.3	10.8	11.3	13.5	10.8	7.5	10.3	10.6	13.3	12.3
Fine silt ...	14.5	12.0	14.5	15.5	10.3	10.0	14.8	20.0	10.5	13.5	13.8	12.5
Clay ...	18.0	18.8	15.5	15.0	15.5	16.3	13.0	14.0	14.0	14.0	14.0	12.5
Hyg. moisture	5.9	4.5	3.1	2.6	2.7	1.9	6.1	4.9	3.6	3.6	3.0	2.7
Ignition ...	19.8	19.0	13.8	7.6	5.6	5.7	24.9	16.9	11.5	10.1	10.1	9.0
R <sub>2</sub> O <sub>3</sub> ...	16.5	16.9	17.6	17.9	18.3	18.8	16.5	16.8	18.2	18.2	18.6	19.1
SiO <sub>2</sub> ...	7.9	5.4	5.1	4.8	4.7	4.3	8.1	6.2	4.6	4.6	4.6	4.5
Exchangeable CaO	0.116	0.063	0.063	0.042	0.038	0.035	0.111	0.071	0.054	0.042	0.036	0.031

	College Farm, Aber					
	(1)	(2)	(3)	(4)	(5)	(6)
Fine gravel	5.2	7.5	7.9	4.3	3.2	3.4
Coarse sand	21.2	18.9	18.0	15.7	10.3	9.1
Fine sand	17.8	18.2	19.9	20.0	15.9	14.7
Silt ...	11.8	11.3	11.8	16.5	14.8	18.0
Fine silt ...	14.5	14.5	16.3	16.3	27.3	23.0
Clay ...	16.3	15.5	15.0	19.8	20.8	25.0
Hyg. moisture	2.0	1.4	1.5	1.4	1.7	1.6
Ignition ...	11.1	5.8	6.0	5.3	4.9	5.7
R <sub>2</sub> O <sub>3</sub> ...	11.9	12.3	13.0	14.7	16.7	18.4
SiO <sub>2</sub> ...	8.6	8.6	8.3	12.9	15.1	14.8
Exchangeable CaO	0.288	0.298	0.287	0.281	0.290	0.274

present A.E.A. official method was decided upon. The dispersion was exactly as in the official method but instead of the five fractions now separated, the old series, including fine gravel as the coarsest fraction, was used.

(2)  $P_2O_5$ ,  $R_2O_3$  ( $Al_2O_3 + Fe_2O_3$ ) and  $SiO_2$  soluble in HCl, according to the conventional method of extraction (20 gm. soil, 70 c.c. 20 per cent. HCl, 48 hr. at  $100^\circ$ ). The  $SiO_2$  was determined by extracting the residue after acid treatment with sodium hydroxide solution of s.g. 1.04.

(3) Exchangeable CaO by the Hissink method.

(4) Citric soluble  $P_2O_5$  by the usual method (100 gm. soil shaken for 24 hr. with 100 c.c. 1 per cent. citric acid).

(5) Nitric soluble  $P_2O_5$ , obtained by extracting 80 gm. soil with 800 c.c. of 0.2N nitric acid, shaking for three hours in an end over end shaker.  $P_2O_5$  was determined in 500 c.c. of the filtrate, equivalent to 50 gm. soil.

In Table I we report the mechanical analyses,  $R_2O_3$ ,  $SiO_2$ , and exchangeable CaO for the successive 3" layers, numbered (1) to (6) of each profile. The data refer to the control plots. We shall allude to them briefly in considering the results at the different centres.

*Penlan, Llangollen.* The soil at this centre, as may be gathered from the mechanical analyses, is a rather heavy silt loam. The heavy texture is mitigated, as generally in N. Wales, by the presence of a considerable proportion of organic matter. The altitude is over 1000 feet and the average annual rainfall 45 inches. From the figures for exchangeable calcium it will be gathered that this soil is distinctly acid. The acid value has been determined by Mr Rice Williams, using Hissink's baryta method, and is for the two top 3" layers equivalent to 3.2 and 2.1 per cent. CaO respectively, so that a high degree of unsaturation is indicated. There are no well-marked pedological horizons, for the soil is immature, but there is a suggestion, in the figures for the sesquioxides, of a downward leaching, which, if it could proceed to a greater degree, would produce a podsolised profile. The soil is thus a potential podsol. It may be mentioned that deeper samples were unobtainable owing to the presence of partially shattered rock. The data illustrating the phosphorus status of the profiles at this centre are given in Table II.

Considering, now, the phosphorus figures and in the first place those for  $P_2O_5$  soluble in 20 per cent. HCl, the general similarity of the control and the 1914 slag profile will be noted. The plot which received slag in 1914 and 1922 resembles the other two except in the top layer, where there is evidence of the slag dressing applied two years previously.

Similar results are seen with the citric and nitric soluble  $P_2O_5$  figures. The effect of the recent slag dressing is plainly seen in the citric figures, and still more strongly in the nitric figures. It also seems possible from the nitric acid figures that the effect of the 1914 dressing is still detectable in the lower layers of the two slag profiles. So far as the upper layers of the soil are concerned it is clear that after ten years all evidence of

Table II. *Phosphorus Status of Penlan Profile.*

		(1)	(2)	(3)	(4)	(5)	(6)
% $P_2O_5$ Sol. in 20 % HCl	Control	0.155	0.123	0.113	0.110	0.094	0.091
	200 lb. $P_2O_5$ 1914	0.156	0.125	0.121	0.113	0.096	0.093
	200 lb. $P_2O_5$ 1914	0.189	0.129	0.107	0.111	0.097	0.094
	and 1922						
% $P_2O_5$ Sol. in 1 % Citric Acid	Control	0.0094	0.0070	0.0073	0.0073	0.0086	0.0085
	200 lb. $P_2O_5$ 1914	0.0100	0.0086	0.0071	0.0075	0.0099	0.0088
	200 lb. $P_2O_5$ 1914	0.0173	0.0075	0.0072	0.0065	0.0095	0.0092
	and 1922						
% $P_2O_5$ Sol. in 0.2N $HNO_3$	Control	0.0023	0.0022	0.0012	0.0010	0.0011	0.0014
	200 lb. $P_2O_5$ 1914	0.0024	0.0021	0.0017	0.0018	0.0016	0.0030
	200 lb. $P_2O_5$ 1914	0.0125	0.0034	0.0018	0.0019	0.0015	0.0026
	and 1922						

the dressing of 200 lb.  $P_2O_5$  in the form of slag has disappeared and that the phosphorus has returned to the status it would have had if no slag had been applied.

*Ffyddion, Caerwys.* The soil at this centre is derived from boulder clay over limestone. There is no calcium carbonate in any of the layers sampled. One may conclude that the soil is of distant origin and that if it ever contained calcium carbonate, long leaching has effected its removal.

The exchangeable calcium is fairly high and one can scarcely consider this soil as suffering markedly from sourness. There is very little evidence of any downward movement of sesquioxides or silica, the

Table III. *Phosphorus Status of Ffyddion Profiles.*

		(1)	(2)	(3)	(4)	(5)	(6)
% $P_2O_5$ Sol. in 20 % HCl	Control A	0.1085	0.101	0.090	0.078	0.062	0.055
	200 lb. $P_2O_5$ 1914	0.115	0.100	0.099	0.079	0.063	0.053
	Control B	0.102	0.093	0.091	0.082	0.059	0.046
	200 lb. $P_2O_5$ 1914	0.142	0.0935	0.091	0.086	0.058	0.049
	and 1922						
% $P_2O_5$ Sol. in 0.2N $HNO_3$	Control A	0.0022	0.0016	0.0012	0.0012	0.0009	0.0006
	200 lb. $P_2O_5$ 1914	0.0024	0.0017	0.0016	0.0017	0.0015	0.0016
	Control B	0.0043	0.0032	0.0027	0.0024	0.0020	0.0013
	200 lb. $P_2O_5$ 1914	0.0138	0.0048	0.0034	0.0044	0.0064	0.0053
	and 1922						

figures for these constituents being fairly constant throughout the profile. In texture the soil is a fairly heavy loam. The altitude is 650 feet and

the mean annual rainfall 35 inches. There was a certain amount of variability in the character of the soil and herbage at this centre. It was therefore decided to take four sets of profile samples, namely (1) 1914 slag, (2) control from adjacent untreated area, (3) 1914 and 1922 slag, and (4) control from adjacent untreated area. The figures are given in Table III. In this and the remaining experiments only 20 per cent. HCl and 0.2N HNO<sub>3</sub> were used for extractions. Comparing the 1914 slag and control it is seen that, apart from a suggestion of a vanishing effect of the 1914 dressing in the nitric acid figures for the lower levels, the two profiles are very similar. In other words, after ten years the plot slagged in 1914 has reverted to the phosphorus status of the untreated soil, as far as the upper layers are concerned.

From a comparison of the plot slagged in 1914 and 1922 with its corresponding control plot one can see evidence of the presence of the more recently applied phosphate in the top layer. The nitric acid figures again bring this out more clearly than the 20 per cent. HCl figures. The lower layers of the 1914-1922 plot show a suggestion of the residual effect of the 1914 applications in the nitric acid figures.

*Maesmynan Hall, Caerwys.* The plots here lie at an altitude of about 450 feet, with a mean annual rainfall of 35-40 inches. The soil is a shaly loam derived *in situ* from Silurian shales. The exchangeable CaO figures indicate a considerable degree of unsaturation. The sesquioxide and silica figures do not suggest any degree of podsolisation. At this centre there was no fresh dressing in 1922, so that the comparison is simply between control and 1914 slag. The two profiles, as in the corresponding cases at the other centres, are similar in the upper layers in their phosphatic figures. Both the 20 per cent. HCl and the nitric acid figures suggest a residual effect in the lower layers. This is scarcely significant in the former case, but in the case of the nitric acid figures there is some degree of probability that the difference between the two plots is not simply normal variation. Though rather outside the scope of the main enquiry, profile figures were obtained for a plot dressed with superphosphate and lime in 1914. The results were similar to those in the slag plot. The effect of the lime on the exchangeable calcium oxide was of course noticeable.

Table IV. *Phosphorus Status of Maesmynan Profiles.*

		(1)	(2)	(3)	(4)	(5)	(6)
% P <sub>2</sub> O <sub>5</sub> Sol. in } Control		0.170	0.163	0.1325	0.129	0.128	0.1225
20 % HCl } 200 lb. P <sub>2</sub> O <sub>5</sub> 1914		0.167	0.163	0.132	0.122	0.130	0.133
% P <sub>2</sub> O <sub>5</sub> Sol. in } Control		0.0066	0.0037	0.0023	0.0023	0.0019	0.0020
0.2N HNO <sub>3</sub> } 200 lb. P <sub>2</sub> O <sub>5</sub> 1914		0.0069	0.0035	0.0020	0.0031	0.0037	0.0039

*Dolwgan, Garn Dolbenmaen.* The soil is a stony heavy loam derived from local drift lying at an altitude of about 800 feet under a mean annual rainfall of 75 inches. A high degree of unsaturation is shown. As there was no re-dressing in 1922 the comparison is, as at the preceding centre, between control and 1914 slag. The general results (Table V) are again exactly similar to those for the other centres. Both HCl and nitric figures show the same reversion to the original phosphorus status and the same suggestion in the lower layers of a residual effect from the 1914 dressing.

Table V. *Phosphorus Status of Dolwgan Profiles.*

		(1)	(2)	(3)	(4)	(5)	(6)
% $P_2O_5$ Sol. in)	Control	0.300	0.295	0.216	0.216	0.218	0.211
20 % HCl {	200 lb. $P_2O_5$ 1914	0.300	0.210	0.215	0.217	0.210	0.211
% $P_2O_5$ Sol. in)	Control	0.0026	0.0014	0.0012	0.0010	0.0011	0.0011
0.2N $HNO_3$ {	200 lb. $P_2O_5$ 1914	0.0026	0.0012	0.0012	0.0017	0.0019	0.0023

*Ffridd, College Farm, Aber.* At this centre a comparison was made between the phosphorus status of a plot treated with slag in 1919 and a corresponding control. The soil is a shaly loam at 1000 feet under a mean annual rainfall of 40–45 inches. A high degree of unsaturation is suggested from the exchangeable CaO figures. Sampled in 1924, this soil shows (Table VI) after five years the residual effect of the slag

Table VI. *Phosphorus Status of Ffridd, Aber. 1000 feet Profiles.*

		(1)	(2)	(3)	(4)	(5)	(6)
% $P_2O_5$ Sol. in)	Control	0.355	0.312	0.302	0.301	0.284	0.259
20 % HCl {	200 lb. $P_2O_5$ 1919	0.377	0.313	0.302	0.300	0.284	0.259
% $P_2O_5$ Sol. in)	Control	0.0063	0.0022	0.0023	0.0024	0.0025	0.0026
0.2N $HNO_3$ {	200 lb. $P_2O_5$ 1919	0.0256	0.0042	0.0029	0.0025	0.0030	0.0027

dressing in the top layers. From the results at other centres, one would have expected that the wastage here would have been more evident. It would appear that in this soil the added phosphate is more stable. The high proportion of HCl-soluble  $P_2O_5$  may be noted in this connexion. High figures are also shown for the sesquioxides. Similar results were obtained from another pair of plots at 800 feet.

*College Farm, Aber.* Profile samples were obtained from one of the grass fields in the lowland portion of the College Farm, Aber. Three plots were examined, namely (1) slag in 1916 equivalent to 143 lb.  $P_2O_5$  per acre, (2) slag in 1925 equivalent to 64.5 lb.  $P_2O_5$  per acre. The results (Table VII) are again in good agreement with those obtained at the other centres and are of special interest, since the field in question has not shown any response to phosphatic dressings.

Table VII. *Phosphorus Status of College Farm, Aber. Lowland Profiles.*

		(1)	(2)	(3)	(4)	(5)	(6)
% $P_2O_5$ Sol. in 20 % HCl	Control	0.249	0.130	0.115	0.098	0.095	0.078
	143 lb. $P_2O_5$ 1916	0.247	0.134	0.115	0.1005	0.104	0.089
	64.5 lb. $P_2O_5$ 1925	0.255	0.131	0.111	0.101	0.093	0.080
% $P_2O_5$ Sol. in 0.2N $HNO_3$	Control	0.0094	0.0071	0.0071	0.0035	0.0021	0.0015
	143 lb. $P_2O_5$ 1916	0.0096	0.0082	0.0098	0.0067	0.0023	0.0012
	64.5 lb. $P_2O_5$ 1925	0.0156	0.0068	0.0073	0.0041	0.0022	0.0012

From the data recorded above it seems evident that in the wet climate of N. Wales with soils deficient in lime, phosphoric acid added to grassland in the form of basic slag does not persist in the soil. At six centres typical of N. Welsh conditions it was found that after ten years soils dressed with slag did not differ from untreated soils and had presumably gone back to their original phosphorus status. Except in the lower layers there was practically no difference between the untreated plots and the plots which had received slag ten years previously. The exact time necessary for this change may be expected to vary according to local conditions of climate and soil, but in all the centres investigated by us it was complete in ten years.

Botanical data collected by Messrs R. Alun Roberts and A. Powell Jones showed that by 1922 the effect of the 1914 slag dressing had completely disappeared. Visible deterioration was evident before this. On the other hand, our analytical data for the plot at Aber which had received slag in 1919, but which had given no effect for the dressing, show that after five years, the added phosphate was still in evidence in the upper layers. As a first inference, then, we may suppose that under the conditions of soil and climate which obtain in our area, the effect of a slag dressing disappears in six to ten years, and that in order to forestall this deterioration in the improved phosphorus status, fresh applications of slag are necessary after, say, five years.

We are unable to decide as to the mechanism of this wastage of added phosphate. There was no evidence at any of the centres of a downward wave of phosphate and we can only conclude that the unstable phosphoric acid sinks directly in the soil. We have not been able to obtain samples of drainage water corresponding with the plots sampled by us. We cannot, therefore, discover whether the leached-out phosphate is lost in the drainage or whether it is reprecipitated at a level lower than 18 inches. The slight increase in the nitric  $P_2O_5$  figures for the 1914 plots as against the control plots in the lower layers does indeed suggest such a precipitation. This point will form the subject of further investiga-

tions. Precipitation of the phosphoric acid might occur either in a more basic stratum or in a stratum containing hydrated sesquioxides.

The approximation in phosphorus status of the 1914 slag plots to that of the controls suggests a division of soil phosphorus in our soils into (a) the natural stable phosphorus compounds which are only subject to negligible wastage by leaching, and (b) the unstable phosphorus compounds of added dressings which are removed fairly rapidly from the surface layers by percolating waters. It may be that the inherent quality of grassland can be correlated with the content of the soil in stable phosphorus compounds.

We should, of course, hesitate to suggest that these results indicate the general instability of added phosphate. They have been obtained in a region where the leaching of the soil by percolating waters is much more intense than under ordinary English conditions. At the same time, conditions such as these obtain over large areas in northern and western Britain. For such areas, it would appear that the permanent improvement of grassland by basic slag can only be achieved by periodical renewal of the dressings.

We would, incidentally, direct attention to the use of 0.2*N* nitric acid, a solvent for "available" phosphoric acid. The differences between the various plots at Penlan were much more evident in the nitric soluble figures than in the figures obtained by the citric acid or hydrochloric acid extraction. Recently added phosphate was clearly shown in the nitric acid figures, but was shown much less certainly by the other solvents. Whilst the use of 0.2*N* nitric acid has as little theoretical justification as the use of 1 per cent. citric acid, an investigation of its applicability in routine soil analysis is desirable. This solvent is already used by many workers abroad and is much more convenient in laboratory practice.

#### SUMMARY.

1. Under the extremely humid conditions obtaining in N. Wales, with soils showing a high degree of base-unsaturation, phosphoric acid applied to permanent grassland as basic slag is fugitive in its effect.

2. From profile analyses it is shown that after six to ten years added phosphoric acid is removed from the surface layers, which revert to their original phosphorus status.

3. It is suggested that the phosphorus of soils may be differentiated into that of the naturally occurring stable phosphates and the phosphorus

of added dressings which is, under N. Welsh conditions, unstable and removable by percolating waters.

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# NOTE ON THE ACTION OF HYDROGEN PEROXIDE ON FARMYARD MANURE IN DIFFERENT STAGES OF DECOMPOSITION.

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G. W. ROBINSON and J. O. JONES<sup>(1)</sup> have shown that humified can be distinguished from non-humified organic matter by the use of 6 per cent. hydrogen peroxide. Humified organic matter is apparently oxidized or rendered soluble by this reagent, whilst structural organic matter is unattacked. It seems reasonable to suppose that a similar distinction might be made in the case of farmyard manure between the amorphous decomposed material and the unaltered fibre of the faeces and litter. In other words, the degree of decomposition of farmyard manure might be determined by a method similar to that suggested for the degree of humification of soil organic matter. It is recognised that farmyard manure differs somewhat from soil organic matter in that the former includes the naturally soluble constituents of the litter, faeces and urine, which are either oxidized completely or rendered soluble in the peroxide treatment. However, they may be regarded as analogous in that both have undergone putrefactive decomposition. In the present paper, humification is used as a convenient term for the processes whereby organic matter is changed to structureless colloidal material and not as implying their exact correspondence with humification in the soil.

## EXPERIMENTAL.

### *I. Profile Samples from College Farm, Aber.*

Samples were required which would best show any correlation between the degree of humification and the known condition and age of the manure. Profile samples were therefore taken from a fairly uniform mass of well-trodden cattle manure produced in a covered shed at the College Farm (November 16th, 1924). This consisted of more than twelve months' accumulation of manure from young cattle fed on hay, straw, roots and cake. It was found to be very uniform and free from any foreign material. The lowest layer with moss litter was not examined.

Above this there were twenty-one inches of moist compressed material in two distinct layers, namely:

(1) Six months' winter accumulation from November to April (inclusive) with plenty of oat straw as litter, comprising the lower layer of twelve inches.

(2) Over six months' summer accumulation from May to middle of November, without litter except the remains of some hay and straw of the rations. This upper layer of nine inches was very dark and could be dug up with a shovel.

Thus, the lower winter accumulation of twelve inches varied in age from fifty-four to thirty weeks, and the upper layer of nine inches without litter consisted mostly of recent droppings, the lowest portion being only ten weeks old. During the summer (thirtieth to tenth week) there was only an occasional animal in the shed, hence the accumulation for this period was negligible. Duplicate samples were taken. Two clearings were made about five yards apart and then slabs of manure, twenty-one inches deep, were cut out down the face of the cutting. These were then horizontally divided into seven three-inch blocks, each weighing about a thousand grams. The blocks of manure were dried and broken up. Representative samples were further ground up and thoroughly dried at 100° C.

The total percentage of organic matter was obtained by determining the "loss on ignition" on 10 gm. of the fine material dried at 100° C. In the peroxide treatments, 5 gm. of fine material were treated in tall 600 c.c. beakers with 200 c.c. of 6 per cent. hydrogen peroxide until frothing ceased. Fresh additions of peroxide were made and the heating continued until no further reaction occurred as shown by the lack of frothing. The contents of the beaker were then made up to about 300 c.c. with water and boiled for twenty minutes, filtered, washed with hot water, and the residue thoroughly dried on the filter in the steam oven. It forms a brittle conical mass of fibrous material which can be conveniently separated from the filter and transferred to a small flat dish. Loss in weight after ignition gives the weight of the structural or non-humified organic matter. Knowing the total amount of organic matter originally present in each sample, the amount oxidized or rendered soluble by hydrogen peroxide can be obtained by difference. The ratio of humified organic matter to total organic matter gives the degree of humification.

The results of the profile samples are set out in Table I. All determinations were made in duplicate.

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Table I. *Profile Samples from College Farm.*

Depth in inches	Total organic matter (%)	Organic matter after treatment (%)	Organic matter decomposed (%)	Degree of humification	Remarks
0-3	77.39	40.50	36.89	47.66	Almost unaltered manure. Without litter
3-6	77.20	39.41	37.79	48.96	
6-9	75.49	37.84	37.66	49.88	
9-12	87.42	56.65	30.77	35.20	Semi-rotten manure. With litter
12-15	87.60	53.54	34.06	38.86	
15-18	87.55	52.40	35.15	40.14	
18-21	85.93	49.22	36.76	42.82	

A sample of dried fresh faeces (cow) contained 87.63 per cent. total organic matter, and 45.74 per cent. organic matter after treatment, giving a percentage humification of 47.81.

It will be seen that for an uniform type of comparatively fresh manure without litter, there was but little variation in the degree of humification. That for fresh faeces averaged 47.81 per cent. compared with an average of 48.83 per cent. for six samples of manure without litter, ranging up to ten weeks old. The agreement between duplicates was good, and there was only a slight increase compared with the value for fresh faeces as we go down into the mass. In the lower strata with litter, there was, as might be expected, a smaller degree of humification. The addition of litter, which consists mainly of structural organic matter, reduced the degree of humification of the total manure from 49.88 per cent. to 35.20 per cent. Analysis showed a continuous increase in humification with depth. As the age of the manure increased from thirty to fifty-four weeks, the corresponding average degree of humification steadily increased from 35.20 per cent. to 42.82 per cent. The increasing rottenness of the straw with the depth as confirmed by analyses was noticeable both in the natural state and on closer examination in the dry state.

The humification value for any fairly uniform mass of manure, as studied by profile analysis of the manure laid down *in situ*, can only be approximate. No allowance can be made for any soluble material that may pass down to the lower levels, thus increasing the apparent degree of humification of the organic matter in the older manure.

Additional non-profile samples of farmyard manure (cattle) were obtained from other farms.

## II. *Coed Howell Farm, Bangor.*

Representative samples of well-rotted manure were taken from four different manure heaps. Three of these samples were almost entirely

free from litter (shippon sweepings) and were over twelve months old. The coarse material of the faeces was very much humified, giving rise to a black slimy mass in the natural state, and becoming brittle when dry. The fourth sample contained some cavings as litter. The results are set out in Table II.

Table II. *Samples from Coed Howell Farm, Bangor.*

Number of sample	Total organic matter (%)	Organic matter after treatment (%)	Organic matter decomposed (%)	Degree of humification	Remarks
1	82.9	30.24	52.66	63.51	Very rotten manure.
2	71.16	25.50	45.66	64.16	Without litter
3	69.70	23.12	46.58	66.83	
4	85.86	42.12	43.74	50.93	With some litter

In the samples of manure containing no litter, the degree of humification varied between 63.51 per cent. and 66.83 per cent. These values for old manure show a marked advance in putrefaction as compared with fresh faeces and comparatively recent droppings, the values for the latter varying between 47.81 per cent. and 49.88 per cent. The addition of some cavings as litter in the fourth sample reduced the humification value to 50.93 per cent.

### III. *Wern Farm, Bangor.*

Five further samples of well-rotted manure were obtained from Wern Farm, Bangor. They all appeared to be very much humified. Samples 1 to 4, which had received small amounts of litter, appeared to be in about the same physical condition as the Coed Howell samples. They were representative of the lowest portions of rather viscous manure heaps made up from byre sweepings. Sample 5, which appeared very much decomposed, was representative of a manure with litter laid down in a calf-box.

It is probable that these samples of old rotten manure indicate about the limit of putrefaction usually met with in farming practice. The results are set out in Table III.

Table III. *Samples from Wern Farm, Bangor.*

Number of sample	Total organic matter (%)	Organic matter after treatment (%)	Organic matter decomposed (%)	Degree of humification	Remarks
1	77.84	29.57	48.27	62.00	Very rotten manure.
2	76.20	26.80	49.40	64.82	With small amounts of litter
3	70.14	23.87	46.27	65.97	
4	75.00	22.43	52.57	70.10	
5	72.40	18.47	53.93	74.49	Rotten calf-box manure. With litter

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' Here again the degree of humification varied between 62·0 per cent. and 70·1 per cent. for four samples, these figures comparing favourably with those in Table II. Sample No. 5 showed 74·49 per cent. humification, this being the maximum value for all the samples dealt with. In these manures the relative small portion that is not oxidised or rendered soluble by peroxide treatment contains much amorphous material and shows a very high ash content. Any earthy material in the manure would be contained in this portion, but would not affect the humification value as the latter refers to the organic matter only.

It will be seen from the foregoing results that there is a good correlation between the degree of decomposition as judged by the appearance and known history of farmyard manure, and its degree of "humification" as shown by the peroxide treatment. The proportion of the organic matter of fresh manure which is in the humified state will depend on the amount of litter present. In the absence of litter about half of the organic matter is originally in the "humified" state. Among the manures examined the humification value was found to vary between 35·0 per cent. and 75·0 per cent. It might be possible to use "degree of humification" as a criterion of the physical condition of farmyard manure.

The work described in the foregoing paper was carried out at Bangor under the direction of Professor G. W. Robinson, M.A.

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# STUDIES ON *BACILLUS AMYLOBACTER*, A. M. ET BREDEMANN.

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(With One Plate.)

## INTRODUCTION.

IN 1894 Winogradsky<sup>(9)</sup> isolated from soil, and described a motile anaerobic bacillus which was capable of fixing free nitrogen, and to which the name *Clostridium Pastorianum* was given. This organism along with a large number of other strains of anaerobic butyric acid bacilli was the subject of an extensive series of researches by Bredemann<sup>(1)</sup> who claimed that the majority (probably all) of the bacilli of this group belong to a single species for which he proposed the name *Bacillus amylobacter*, A. M. et Bredemann. Bredemann was able to show that all members of this group become capable of fixing nitrogen after they have been cultivated for some time in soil. In the course of his investigations he noticed that many of his cultures showed a tendency to produce coccoid bodies (the so-called "microoidia" of Arthur Meyer) which differed from the typical bacilli not only in morphology but also in their ability to grow aerobically and in their failure to produce gas from sugars. This observation immediately suggests the possibility that the cocci were present as contaminants in the original cultures, especially in view of the difficulties frequently experienced in preparing pure cultures of anaerobic bacteria. With regard to the purity of his cultures Bredemann states: "Ich möchte jedoch ganz besonders hervorheben dass ich mich von der absoluten Richtigkeit dieser Erscheinungen an ganz einwandsfreien Reinkulturen wiederholt überzeugt habe." No direct experimental proofs of this contention are however given in his paper which, so far as the microoidia are concerned, is of a preliminary nature and contains no more detailed description of these organisms. Coccoid bodies have also been observed in cultures of bacilli of this group by H. Buchner<sup>(2)</sup> and van Tieghem<sup>(8)</sup>. Winogradsky<sup>(10)</sup> and Haselhoff and Bredemann<sup>(5)</sup> record similar observations but consider the cocci to be incapable of reproduction. With the exception of Bredemann none of these workers succeeded in obtaining the cocci in pure culture.

In view of the recent work of Löhnis and Smith<sup>(6, 7)</sup> on the life

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cycles of the bacteria Bredemann's observations, if correct, acquire an increased importance. An attempt has therefore been made to confirm and extend them, and in particular:

- (1) To establish the relationship of the coccoid form to the bacillus.
- (2) To study in greater detail the differences between these two types.
- (3) To determine whether the coccus is, like the bacillus, capable of fixing nitrogen. If this is not the case and if the bacillus passes readily into the coccoid form, it is obviously important to study the conditions under which this transformation takes place.

### EXPERIMENTAL.

Five strains of the motile butyric acid bacillus were isolated from five different soils. About one gram of soil was placed in each of a number of test tubes containing sterile one per cent. dextrose solution. The medium was then heated to 80° C. for ten minutes and incubated anaerobically in a McIntosh and Fildes jar at 37° C. After 48 hours, vigorous gas formation took place and isolations were made by plating anaerobically on 0.5 per cent. dextrose agar from tubes of fermented dextrose solution which had been again heated to 80° C. for ten minutes. After 24 to 48 hours' incubation at 37° C. small colonies appeared on the plates and the medium was broken up by gas bubbles. Selected colonies were purified by repeated plating, the inoculum being heated to 80° C. before each plating. A sixth strain of the bacillus was obtained from milk by heating the milk to 80° C., incubating anaerobically at 37° C. and plating as already described. After purification the cultures were transferred to tubes of sterile milk containing calcium carbonate, in which they remained viable for at least two months. No difficulty was experienced in maintaining stable cultures in this medium provided that they were heated to 80° C. before each transfer. If the preliminary heating was omitted, the cultures generally failed to produce gas; this is in accordance with Bredemann's experience.

The characteristics of the cultures isolated were as follows: the organism was a straight rod with rounded ends, 3-8  $\mu$  long by 1  $\mu$  broad. It was actively motile; motility could be demonstrated in a drop of liquid medium examined under a cover glass on an ordinary slide. The organism was Gram positive and produced an ovoid central or sub-terminal spore, usually wider than the cell. It grew well at 30° to 37° C. and slowly at 22° C. and was destroyed by boiling for three minutes in dextrose bouillon. It was a strict anaerobe.

On ordinary meat-extract agar minute whitish colonies were produced. Growth was more vigorous on 0.5 per cent. dextrose agar and was accompanied by splitting of the medium due to gas formation. The colonies were greyish and translucent.

On ordinary meat-extract gelatine no growth was obtained, but on 0.5 per cent. dextrose gelatine greyish granular colonies with hair-like outgrowths were produced. The gelatine was not liquefied.

In bouillon a very scanty growth was observed, while in 1 per cent. dextrose bouillon growth was vigorous and was accompanied by gas formation.

In milk an acid frothy curd was formed; the curd contracted and expressed a considerable quantity of whitish whey.

On potato growth was abundant and white.

All strains fermented dextrose, levulose, galactose, saccharose, maltose, lactose, xylose, dextrin, inulin, mannitol, sorbitol, and salicin dissolved in peptone water, producing acid and gas in each case. Dulcitol and adonitol were not fermented.

The bacillus was grown in two per cent. dextrose and one per cent. peptone dissolved in Winogradsky's mineral solution containing a known quantity of calcium carbonate till fermentation ceased. The acids produced were liberated by means of the calculated quantity of sulphuric acid. The application of Dyer's<sup>(1)</sup> qualitative tests indicated the presence of butyric and acetic acids. A Duclaux distillation showed that these were present in the molecular proportion of approximately 1.75 to 1. The residue from the distillation was extracted with ether and the extract failed to give a reaction with Uffelmann's reagent.

In peptone water the bacillus did not produce indol.

It was non-pathogenic for guinea-pigs inoculated intraperitoneally.

In order to ensure that the cultures of the bacillus were pure they were replated several times. In the jar in which the plates from the fifth plating were incubated complete anaerobiosis was not secured; the reduced methylene blue solution became faintly blue during incubation. When the jar was opened numerous colonies about 3 to 4 mm. in diameter were observed on plates from two different strains. The colonies occurred in groups towards one side of the plate and in addition to being larger than those of the butyric acid bacillus they differed from the latter in being thick, white and opaque. The growth from these colonies was replated aerobically as well as anaerobically when it was found that a more vigorous growth could be obtained under aerobic conditions than could be secured anaerobically. The cultures were purified by replating



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aerobically on dextrose agar. Fourteen strains of this organism have been isolated and studied.

The organism proved to be a coccus, 0.75 to 2 $\mu$  in diameter which occurred singly, in pairs, in fours, in short chains and in irregular groups. When the cells occurred in pairs or in fours they were frequently bean-shaped. The organism was Gram positive, non-motile and non-sporing. It grew well at 30° to 37° C., slowly at 22° C. and not at all at 50° C. and was destroyed in dextrose bouillon at 58 to 60° C. in ten minutes. It was a facultative anaerobe.

On ordinary agar a white growth was formed. On dextrose agar growth was abundant, opaque, porcelain-white and glistening, while the colonies were white, slightly raised and round with uniform entire margin. No gas was produced.

On ordinary gelatine a white glistening growth was formed on the surface of the medium and a greyish papillate growth in the stab. The majority of the fourteen strains examined failed to liquefy gelatine; five strains produced a very slow liquefaction.

In bouillon a dense turbidity was formed and an abundant slimy sediment accumulated at the bottom of the tube. The medium became alkaline.

Eight of the fourteen strains produced an acid-rennet curd in milk, generally after several days' incubation. A considerable quantity of a whitish whey was expressed. No gas was formed. Six strains failed to curdle milk but rendered it slightly acid.

On potato a glistening white growth was formed and the medium was not discoloured. Some strains produced a more abundant growth than others.

Acid but no gas was produced in dextrose, levulose, lactose, maltose, and saccharose dissolved in peptone water. Adonitol, inositol and inulin were not fermented even after one month's incubation. A weak and delayed fermentation of dulcitol and sorbitol was observed in the case of one strain only. The remaining strains failed to attack these alcohols.

The volatile acids produced from dextrose were butyric and acetic in the molecular proportion of approximately 1 to 6.4. The residue from the Duclaux distillation gave a positive reaction with Uffelmann's reagent.

Indol was not produced in peptone water.

In soil extract containing two per cent. dextrose, 0.05 per cent. dipotassium phosphate and excess of calcium carbonate no nitrogen was fixed.

The organism was non-pathogenic for guinea-pigs inoculated intraperitoneally.

Two of the strains examined produced an orange-coloured growth on gelatine, agar and potato but were otherwise similar to the white strains already described.

The cocci are similar to that described by Bredemann, not only in morphology, but also in their ability to grow aerobically and in their inability to produce gas from sugars. They possess in addition a number of other characteristics, not recorded by Bredemann, which clearly distinguish them from the bacillus, and they would ordinarily be classified as distinct species. The white and orange strains correspond respectively to *M. candicans* Flüge and *M. aurantiacus* Cohn, except that some of the cultures curdle milk. An attempt has been made to determine whether they owe their origin to contamination or are derived from the bacillus.

The possible sources of the coccus as a contaminant are (1) the media used, (2) the air during the inoculation of the media, (3) the apparatus, (4) the original cultures of the bacillus. It has been found comparatively easy to exclude sources 1 to 3. The experiments have been repeated several times using uninoculated media incubated along with media inoculated with cultures of the bacillus and in all cases the coccus has only appeared in the latter.

The absence of a contamination in the original cultures of the bacillus is more difficult to prove. It has already been recorded that the original cultures were purified by plating and that the coccus was first observed in cultures which had already been plated four times. The plating of the original cultures was continued, but the coccus still appeared in cultures which had been plated twenty or more times. In the plating method employed the medium was inoculated before it was poured into the Petri dishes. For most organisms this method is undoubtedly superior to that in which the inoculum is smeared over the surface of media already solidified in the dishes. In the case of gas-producing organisms, however, a complication arises from the fact that the medium in poured plates is liable to be broken up by gas formation. A considerable quantity of liquid is thus pressed out of the agar and this may allow of the spread of the growth from one colony to another. It must also be remembered that the suspected contaminant is a facultative anaerobe and is therefore capable of growing under strictly anaerobic conditions. Theoretically, therefore, there are objections to placing entire reliance on the plating method for securing pure cultures of the butyric acid

bacillus. Attempts were made to obtain single-cell cultures of the bacillus by Burri's Indian ink method, but in all cases it was found impossible to secure growth of the single cells. This is in harmony with the findings of Dorner<sup>(3)</sup> who only succeeded in getting about 1 per cent. of the cells isolated to grow, even when special media were employed.

A careful and extended microscopic examination has invariably failed to reveal the presence of typical cocci in the stock cultures of the bacillus. Controlled experiments were performed in which the cultures, which had been examined microscopically, were plated and the numbers of colonies of the coccus which developed were counted. A loopful of the condensation water from a vigorously-growing culture of the bacillus on a dextrose agar slope was added to a small quantity of boiled and cooled sterile water. Three loopfuls of the suspension were spread over a square of 1 cm. side on a glass slide. The film was dried, fixed and stained with dilute fuchsin and systematically searched with a 1/12-inch oil-immersion objective using a mechanical stage. No cocci were observed, but when one loopful of the suspension was used to inoculate each of a number of dextrose agar plates from 5 to 600 colonies of the coccus appeared on each plate after incubation. This experiment was repeated fourteen times with similar results.

In the cultures of the bacillus small ovoid or spherical bud-like processes were sometimes observed attached to the cells. Such bodies have been described by Löhnis and Smith as gonidia. They may become separated from the rods in which case they bear a certain resemblance to cocci but occur singly. They are not however sufficiently numerous to account for the numbers of coccus colonies observed. For example, in one experiment 5 and in another 10 of these bodies were found in three loopfuls of material which on inoculation (one loopful) into dextrose agar yielded 600 and 50 coccus colonies respectively.

In describing the first isolation of the coccus reference has already been made to the fact that the organism appeared on plates which had been incubated under incompletely anaerobic conditions. No growth of the coccus from cultures of the bacillus has ever been obtained on plates incubated under strictly anaerobic conditions though it has frequently been observed that the coccus appeared on anaerobic plates incubated in a jar which had been opened from time to time, or on plates which had been incubated aerobically after a period in a McIntosh and Fildes jar. When plates inoculated with the bacillus were incubated aerobically no growth was obtained. Carefully controlled experiments were carried out in which equal inoculations were made from the same culture of

the bacillus into a series of tubes containing melted dextrose agar. The tubes were poured into sterile Petri dishes and incubated at 37° C., some aerobically, some anaerobically and some under partially aerobic conditions. Partially aerobic conditions were secured by placing the Petri dishes in a Bulloch's jar and connecting it to a water pump for about half-an-hour. This experiment has been frequently repeated and the coccus has been obtained twelve times in the plates incubated under partially aerobic conditions. No growth has ever been obtained aerobically. In the plates incubated anaerobically colonies of the bacillus developed. Frequently the coccus appeared on these plates after the jar in which they were incubated had been opened once or twice.

These experiments seem to indicate that, though the coccus grows well aerobically, it only develops from the bacillus under partially aerobic conditions. In addition they provide further evidence that the cultures of the bacillus were pure. It seems improbable that, if the coccus were present in the latter, it would not develop when they were plated aerobically. The possibility of inhibition of the growth of the coccus by the bacillus must however be considered. To test this a trace of growth from a coccus culture was added to a culture of the bacillus and the mixture was plated aerobically on dextrose agar. No indication of inhibition of the coccus was observed, as numerous colonies appeared on the plates after 24 hours' incubation.

Further evidence of the relationship of the coccus to the bacillus was obtained as a result of experiments based upon the difference in heat resistance of the two organisms. The coccus was destroyed at 60° C. in ten minutes while an exposure of three minutes to a temperature of 100° C. was necessary to kill the bacillus. Cultures of the bacillus were therefore heated at 80° C. for ten minutes and plated under partially aerobic conditions or anaerobically in jars which were frequently opened. Cocci have been obtained in 18 of these experiments. Dextrose agar plates inoculated from heated cultures and incubated aerobically yielded no growth.

#### DISCUSSION.

In order to establish the connection of the coccus with the butyric acid bacillus everything depends upon the exclusion of possible sources of contamination because, if the contamination hypothesis can be ruled out, the only explanation of the appearance of the coccus in cultures of the bacillus is that it forms a part of the life-cycle of the latter organism. The control experiments performed with uninoculated media, etc., satisfactorily exclude external sources of contamination. Possible

contamination of the bacillus cultures is adequately dealt with in the other experiments described. The experiments with microscopic control provide good evidence, unless one assumes that the coccus arises from bodies so small that they escape detection by microscopic examination. It is extremely difficult to explain why, if the bacillus cultures originally contain the coccus as a contamination, the latter does not develop on plates incubated aerobically. Still more convincing is the evidence provided by the experiments carried out with heated material, especially when it is stated that the stock cultures of the bacillus from which inoculations have been taken have now been transferred 12 to 14 times and each time the inoculated cultures have been heated to 80° C. for ten minutes—a temperature well above the thermal death-point of the coccus. Attention is also directed to the fact that the coccus cultures isolated are all of the same type. The variation in gelatine liquefaction is of little importance as many cocci produce a very slow liquefaction of gelatine. It is interesting to note in this connection that the cocci obtained by Löhnis and Smith<sup>(7)</sup> from cultures of *Azotobacter* showed the same type of variation. To differences in behaviour in milk little significance is attached, as cocci of this type rapidly lose the capacity for curdling milk. It is further noteworthy that cocci have been obtained from all six strains of the bacillus studied. Some strains of the bacillus however yield coccus cultures more readily than others.

The study of the life cycle of the motile butyric acid bacillus is being continued by one of us (A. C.), who has already obtained cultures of four types of aerobic bacilli believed to be derived from that organism. These include a small cocco-bacillus type, slender and stout non-sporing rods and a sporing bacillus type. Descriptions of these will be given in future papers along with evidence of their relationship to the butyric acid bacillus. In the meantime attention is drawn to the fact that three of these types have already yielded coccus cultures.

We are indebted to Mr Richard Muir, Pathology Dept., University of Edinburgh for the microphotographs which accompany this paper.

#### SUMMARY AND CONCLUSIONS.

(1) An aerobic coccus has been obtained from cultures of the motile butyric acid bacillus under conditions which exclude the possibility of contamination.

(2) Descriptions of the coccus and the bacillus are given.

(3) The coccus does not fix nitrogen in soil extract containing dextrose.



Fig. 1. *Bacillus amylobacter*. 2 days old culture on dextrose agar.  $\times 1000$ .



Fig. 2. Coccus obtained from *Bacillus amylobacter*. 31 days old culture on ordinary agar.  $\times 1000$ .



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# THE INFLUENCE OF THE STAGE OF LACTATION AND THE BREED OF THE COW ON THE YIELD AND QUALITY OF THE MILK.

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(With One Text-figure.)

THE principal factors<sup>1</sup> influencing the yield and quality of milk are (1) stage of the lactation period, (2) breed of the cow, (3) interval between milkings, (4) age of the cow, (5) individuality of the cow, (6) efficiency of the milker, (7) temperature and weather conditions, (8) health of the cow, (9) feeding.

Experiments conducted upon the milk yielded by a single cow kept under standard conditions gave such variable results with no apparent reasons for the fluctuations that this method of procedure was abandoned. Thus the following are a few results illustrating the variations observed for the 16th, 17th, 18th, 19th and 20th day since calving. The fat percentages were respectively 3.51, 3.72, 3.94, 3.62 and 3.83, whilst the corresponding percentages for solids-not-fat were 9.11, 9.21, 8.88, 9.17 and 9.06, giving the total solids respectively as 12.62, 12.93, 12.82, 12.79, 12.89 per cent.

Similar results were obtained by Eccles and Shaw<sup>2</sup>, and even analyses of composite samples representing each week's or month's milk from an individual cow gave great variations, and curves showing the yields, percentages of fat and solids-not-fat were very irregular.

It was therefore concluded that the influence of the period of lactation on the yield and quality of the milk of cows of the various breeds could only be studied by making use of many thousands of experiments made as far as possible under the same conditions. The analyses of the milks of the cows exhibited at the past forty-eight annual Dairy Shows

<sup>1</sup> See Mackintosh, *Trans. Highland and Agric. Soc. Scotland*, 1925, and Eccles and Shaw, *U.S. Dept. of Agric. 1913, Bull. 155*.

<sup>2</sup> *U.S. Dept. of Agric. 1913, Bull. 157*.

held by the British Dairy Farmers' Association between 1876 and 1925 seemed eminently suited to this purpose for the following reasons. Owing to the enormous number of cows considered, the individuality and variation in health of the single cow becomes eliminated; also it may be justly contended that the kind and quality of the food would naturally be, as far as possible, that most suited to the animal. Further, the efficiency of the milkers in such contests is probably at a maximum and since the shows have been held over a large number of years, any variations in weather and abnormal show-yard conditions will tend to be neutralised. From the above it will be observed that five of the factors influencing the yield and quality of the milk are eliminated and study becomes possible of the remaining four: (1) stage of lactation period, (2) breed of cow, (3) interval between the milkings, (4) age of cow.

The records in the hands of the British Dairy Farmers' Association give the breed, stage of lactation, age of cow, quantities of milk given at the consecutive morning and evening milkings of one day, and analyses of all such samples of milk for 3283 specially selected cows. The cows are usually carefully prepared for show purposes, and their yields of milk may possibly have been higher than is the normal case with commercial cows at the same stage of lactation. It should also be observed that the period of lactation studied in this investigation is not a complete one, and comprises the first 210 days.

The influence of the age of the cow is receiving separate attention and a further communication on this subject will be made in the immediate future. In this paper, attention will be concerned with the first three factors given above.

#### GENERAL RESULTS.

In the first instance, the 6566 analyses were dealt with as a whole without reference to the particular breed of the cows. The averages obtained for the yields of milk, percentages of fat and solids-not-fat at the morning and evening milking at the stated stages of the lactation period are given in Table I.

The validity however, of these results as true averages of all breeds of cows depends on an even distribution of the breeds throughout the lactation period under consideration. The actual distribution is given in Table II, and it will be seen that the Dairy Shorthorn breed predominates in the early lactation period, whilst the Jersey cows are present in relatively greater proportion in the later stages. Thus it was

concluded that each breed must be dealt with separately, and Fig. 1 shows the result obtained in the case of Jersey cows (Table VII), the

Table I. *All breeds.*

Days since calving	Morning milk			Evening milk			Day's milk		
	Yield	Fat	Solids- not-fat	Yield	Fat	Solids- not-fat	Yield	Fat	Solids- not-fat
15	23.7	3.98	9.34	20.8	4.60	9.21	44.5	4.26	9.29
20	23.5	3.82	9.26	21.3	4.49	9.12	44.8	4.13	9.21
30	23.9	3.68	9.18	21.8	4.35	9.03	45.7	3.93	9.11
40	23.3	3.66	9.14	20.2	4.30	9.03	43.5	3.96	9.08
50	21.3	3.69	9.10	19.0	4.34	9.05	40.3	3.99	9.08
60	20.6	3.81	9.15	18.9	4.31	9.05	39.5	4.05	9.13
70	19.9	3.85	9.21	18.1	4.41	9.07	38.0	4.08	9.14
80	19.7	3.75	9.18	17.9	4.40	9.06	37.6	4.06	9.15
90	19.9	3.85	9.18	18.0	4.46	9.06	37.9	4.09	9.12
100	19.7	3.93	9.17	17.3	4.52	9.06	37.0	4.20	9.12
110	18.9	4.07	9.20	16.8	4.75	9.08	35.7	4.38	9.16
120	18.8	4.27	9.20	16.7	4.88	9.09	35.5	4.55	9.14
130	18.5	4.37	9.16	16.4	4.88	9.07	34.9	4.61	9.15
140	17.6	4.45	9.22	15.5	4.84	9.11	33.1	4.63	9.15
150	17.2	4.48	9.20	14.8	4.84	9.13	32.0	4.64	9.16
160	17.0	4.48	9.22	14.6	5.03	9.16	31.6	4.74	9.24
170	16.9	4.50	9.28	14.7	5.13	9.21	31.6	4.79	9.24
180	16.4	4.72	9.25	14.7	5.04	9.15	31.1	4.81	9.21
190	15.8	5.01	9.23	13.5	5.39	9.09	29.3	5.18	9.17
200	15.6	5.07	9.26	13.1	5.46	9.08	28.7	5.24	9.17
210	15.7	5.08	9.29	14.1	5.50	9.19	29.8	5.27	9.24

curves being drawn as evenly as possible through the actual average points.

#### DISCUSSION OF THE RESULTS.

The results for the Jersey breed are represented in the figure, and a general discussion of the results is applicable to all breeds.

*Fat.* From the figure it will be observed that the percentage of fat in both the morning and evening milk at first decreases until about the 40th day when the minimum is reached. From that day, the average percentage of fat shows a regular increase as the stage of lactation advances. The total range for the morning milk is from 4.32 per cent. fat (40th day) to 5.65 per cent. (210th day), and for the evening milk from 5.30 per cent. fat (40th day) to 6.14 per cent. (210th day).

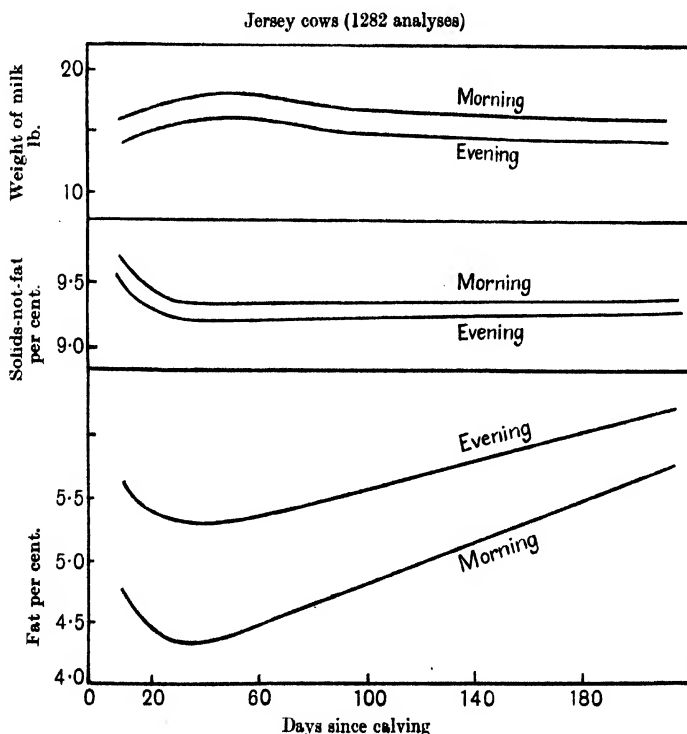
The morning and evening milkings occurred at intervals of 13 and 11 hours respectively and it will be noted that the curves for the percentages of fat are not absolutely parallel. At first (15 days) the percentage of fat in the evening milk exceeds that in the morning milk by 0.83 per cent., while at 30 days the difference is 0.98 per cent. After the minimum percentage of fat has been passed at about the 40th day,

however, this difference diminishes. Thus at the 120th day the difference is reduced to 0.74 per cent. and at the 200th day to 0.52 per cent.

Table II. *Breeds and number of cows.*

	Lactation period in days																						Total
	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220		
Jersey	26	34	44	40	44	48	39	27	26	31	31	27	32	27	29	34	30	30	19	10	13	641	
Guernsey	22	25	34	24	23	29	12	8	9	15	4	11	9	17	11	6	12	7	7	2	4	291	
Dairy Shorthorn	193	284	209	103	74	55	37	33	24	16	19	11	11	6	12	7	10	2	4	1	2	1114	
Red Poll	19	43	62	57	47	33	21	20	9	8	7	4	8	5	4	13	5	4	2	0	3	374	
British Friesian	23	35	30	18	21	8	8	3	7	8	3	5	3	4	3	4	0	1	1	1	1	187	
Devon	7	4	14	7	3	1	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	39	
South Devon	1	4	8	3	5	1	2	8	3	0	1	1	5	5	4	1	2	0	0	0	1	55	
Lincoln Red	19	38	32	38	17	11	4	6	4	1	3	3	3	6	1	1	1	1	0	0	0	189	
Dexter	2	4	3	6	4	3	7	2	5	3	2	3	3	4	5	7	3	4	5	3	2	80	
Kerry	11	25	22	28	17	11	5	4	8	2	3	3	5	6	7	5	3	3	2	1	0	171	
Ayrshire	32	32	31	15	9	3	5	4	1	2	1	3	1	1	1	0	1	0	0	0	0	142	
Total	355	528	489	340	264	208	140	115	96	86	74	71	80	82	78	78	66	53	41	18	26	3283	

*Solids-not-fat.* In the case of the percentages of solids-not-fat in the morning and evening milk, the figure shows that similar changes are passed through as the stage of the lactation period advances. There is first a



fall in the percentage to a minimum at about the 40th day and then a steady rise, the curves for the morning and evening milk being almost parallel. The ranges of the results are more restricted than for the fat, thus the solids-not-fat in the morning milk varies from 9.34 to 9.36 per cent., and in the evening milk from 9.20 to 9.22 per cent. on the 40th and 210th days respectively.

*Yield.* The average yield of milk at first increases and reaches a maximum at about the 45th day subsequent to calving. From that time there is a steady decrease as the time advances, which gives an almost straight line. The curves for the morning and evening yield are parallel and show an average difference of 2 lb. of milk. Any appreciable increase in the milk production is accompanied by a reduction in the percentage of fat<sup>1</sup>. However, according to Mackintosh<sup>2</sup> the percentage of fat falls to its lowest at the time of the highest yield of milk. From the values given in the tables it will be observed that as a rule the minimum percentage of fat occurs some time after the date of the maximum yield of milk<sup>3</sup>.

*Interval between milkings.* As a rule, the shorter the time between milking, the smaller is the yield of milk for that milking and also the percentage of solids-not-fat in that milk, but the greater is the percentage of fat<sup>4</sup>. This statement is based on the general experience observed when cows are milked at uneven periods, the longer period being during the night. No evidence has been obtained when the longer interval occurred during the day. The percentage of fat has received special attention. Thus it was found that a herd of 20 cows when milked at the following intervals gave the average results shown in Table III.

Table III.

Interval since last milking, hours	Yield, lb.	Fat, %	Remarks
12	16.2	3.54	Morning milk
12	15.7	3.73	Evening „
	31.9	3.64	Day's „
13	16.9	3.49	Morning „
11	14.7	3.85	Evening „
	31.6	3.66	Day's „
14	17.8	3.42	Morning „
10	13.4	4.07	Evening „
	31.2	3.70	Day's „

<sup>1</sup> See Hittcher, *Landwirthschaftliche Jahrbücher*, 1894, **23**, 873.

<sup>2</sup> *Loc. cit.*

<sup>3</sup> Cp. also Tocher, p. 16, *loc. cit.*

<sup>4</sup> See Fleischmann, *Landwirthschaftliche Jahrbücher*, 1891, **20**, sup. 2.

Results of a somewhat similar character have been recorded by Buckley<sup>1</sup>, although Eccles and Shaw<sup>2</sup> state that when a cow is milked twice daily at intervals of equal length there is only a small variation in the average fat content of the milk and that the variations found under these conditions seem to depend upon the individuality of the cows.

From Table III, it will be observed that milking at regular intervals of 12 hours did not give equal quantities of milk containing the same percentage of fat. The evening fat result was higher than that for the morning. Gilchrist<sup>3</sup> draws attention to the effects of changes of temperature on the quality of milk, and Lloyd<sup>4</sup> has shown that a low fat content in the morning milk often followed a sudden fall in temperature during the night; and that a rise in temperature during the day tended to cause a higher percentage of fat in the evening milk. He also states that cows acclimatised themselves in a striking manner to a new constant temperature whether it was high or low.

Probably variations in temperature are not the sole cause of the fluctuations in the percentage of fat in the milk; but the influence is not in this case self-eliminating on taking averages of large numbers of analyses of morning and evening milks, since at the Dairy Show the cows are undoubtedly subjected to a fall of temperature during the night.

It is therefore necessary in considering the curves shown in the figure for the fat in the morning and evening milk to note that the two distinct curves are not solely due to the different intervals between the respective milkings but also among other possible causes to the respective variations in temperature between the milkings.

No figures have been obtained with regard to the solids-not-fat in the morning and evening milk of a herd milked at different intervals.

The effect of the stage of lactation in the milk of ten other breeds of cows was studied and the shapes of the curves obtained for the various breeds were found to correspond very closely to those indicated in Fig. 1 for the Jersey breed. The results are given in Tables VII to XVII and where insufficient data have been available to make absolutely definite values possible, the figures have been obtained from the probable position of the curves. The figures deduced in this manner are given in italics in the tables.

<sup>1</sup> *Some Observations on the Butter Fat in Cows' Milk*: Printed for private circulation.

<sup>2</sup> *U.S. Dept. of Agric. 1913, Bull. 157, 14.*

<sup>3</sup> *Report on Dairy Investigations at Offerton Hall, Durham, 1909, p. 16.*

<sup>4</sup> *Journ. British Dairy Farmers' Assoc. 1916, 30, 71.*

There are further influences on the quantity and quality of milk for the lactation period, which have received no direct consideration in the paper.

*The actual date of calving.* Since the Dairy Show organised by the British Dairy Farmers' Association has always taken place in October, it follows that records for the early part of the lactation period relate to cows which have calved in the fall of the year and that as the stage of the lactation advances so the date of calving retreats through the year. It has been shown<sup>1</sup> that a difference exists between the results of spring and fall cows although this is not great, but it is obvious that such differences as exist will not be eliminated in the results given in this paper.

*The farrow cow.* The British Dairy Farmer's Association possess no records stating whether the cow was farrow or in calf at the time of the milking trials, although Cooke<sup>2</sup> and Eccles and Shaw<sup>3</sup> have shown that this condition slightly influences the behaviour of the cow throughout the lactation period.

*The yield of butter fat and solids-not-fat.* From the curves obtained for the various breeds, the actual weights in lb. of fat and solids-not-fat for each day of the lactation period were calculated, and the results given in Tables VII–XVII. From these tables it will be observed that the total weight of fat given by the heavier milking breeds at first decreases rapidly and then remains almost constant until the later stages of the lactation period when there is a tendency to rise slightly.

One peculiarity will be observed in the actual weight of fat in the morning and evening milks of certain breeds. At first the amount of fat is greatest in the evening milk, but at about the 110th day the morning and evening yields of fat become equal and subsequently the morning yield of fat exceeds the evening. This apparently does not hold for the following breeds, Guernsey, Red Poll, British Friesian, Dexter.

However, even in these cases, in which the weight of fat in the evening milk never exceeds that of the morning, the difference between the weights given at the two milkings increases towards the end of the lactation period. This is due to the fact that whereas (as previously stated) the rate of decrease in the yield of milk is the same for both morning and evening milkings during the period studied, the increase

<sup>1</sup> See Cooke, *Vermont Agricultural Experiment Station, Sixth Annual Report, 1892*, and Hills, *ibid. Ninth Annual Report, 1895*.

<sup>2</sup> *Loc. cit.*

<sup>3</sup> *Loc. cit.*

in the percentage of fat is less rapid in the evening than in the morning in every breed.

In every case the yield of solids-not-fat increases until about the 30th day, and then decreases. The morning yield of solids-not-fat exceeds that for the evening throughout the lactation period studied. The percentage of fat in the total solids falls to a minimum early in the lactation period and then increases as the lactation period advances. This follows the fat percentage curve<sup>1</sup>.

The amount of milk containing 1 lb. of fat at first increases and then diminishes steadily throughout the lactation period.

Reference may be made to the results and curves recently published by Tocher<sup>2</sup>. On page 75 is given a curve showing, as the result of 676 analyses, the regression of butter fat on weeks in milk. The minimum percentage of fat is reached between the 14th and 16th week in milk, whereas the authors have obtained in most cases a minimum considerably earlier in the lactation period. This discrepancy appears to be due to the fact that of the 676 analyses studied by Tocher, 341 were of milks of Ayrshire cows, and the curves will therefore be dominated by the characteristics of the Ayrshire breed. This breed was found by the authors to give a minimum percentage of fat as late as the 16th week after calving.

Speir<sup>3</sup> records the lowest fat content as early as the 4th week, but Trunz<sup>4</sup>, Linfield<sup>5</sup> and Eccles and Shaw<sup>6</sup> give the minimum at about the 12th week.

A similar discrepancy occurs between Tocher's curves<sup>7</sup> and the authors' values (see Tables VII-XVII) for the regression of the percentage of solids-not-fat on days in milk. The minimum observed by Tocher was between the 17th and 21st week in milk whereas the minimum obtained by the authors varied from about the 6th to the 18th week after calving and depended upon the breed.

The curves for the yield of milk show better agreement, the maximum yield being observed in each case early in the lactation period.

*Composition of the milk.* Complete analyses of the milk samples for the whole lactation period have not been obtained, but in Table IV are

<sup>1</sup> Cp. Hinchcliff, *loc. cit.*

<sup>2</sup> *Variations in the Composition of Milk*, H.M. Stationery Office, 1925.

<sup>3</sup> *Trans. Highland and Agric. Soc. of Scotland*, 1905, **17**, 182.

<sup>4</sup> *Zeit. Physiol. Chem.* 1903, **39**, 390.

<sup>5</sup> *Utah Agric. Exp. Station*, 1900, *Bull.* **68**.

<sup>6</sup> *Loc. cit.*

<sup>7</sup> *Loc. cit.* p. 75.



given the average analyses of the milks of 20 Jersey cows which have calved at the times stated.

Table IV.

		Days since calving		
		20-40	90-110	180-220
Fat	%	5.02	5.24	5.73
Protein	"	3.98	4.11	4.45
Ash	"	0.77	0.77	0.79
Sugar	"	4.91	4.90	4.84
Total solids	"	14.68	15.02	15.81

In considering the above results, it must be observed that no records could be obtained of the age of the cows and any large variation in this factor may have had a slight influence on the data given in Table IV<sup>1</sup>. The results, however, show that the protein and fat percentages tend to increase with the progress of lactation<sup>2</sup>.

The sugar is fairly constant<sup>3</sup> although reports have been made stating it to be the most variable constituent<sup>4</sup>. The ash percentage is the most constant although the final value shows a slight increase.

*The influence of the breed on the quantity and quality of the milk.* In the foregoing sections, the influence of the period of lactation on the quality and quantity of the milk has been mainly considered.

For the purpose of studying the influence of the breed of the cow a comparative summary is given in Table V. In the first two columns of this table are given respectively the times to attain the maximum yield of milk and the minimum percentage of fat.

Table V. Comparison of breeds.

Breed	Day of max. milk yield	Day of min. fat percentage	15th to 200th day after calving						From 100th to 200th day after calving	
			Total daily yield lb.	Average daily yield lb.	Average percent. fat	Average weight fat	Average percent. solids-not-fat	Average weight solids-not-fat	Decrease in yield %	Increase in fat content %
Jersey	45	40	5817	31.4	5.18	1.62	9.30	2.91	5.74	9.95
Guernsey	30	35	5550	30.0	4.88	1.46	9.29	2.78	14.5	15.3
Dairy Shorthorn	30	90	7003	41.1	3.78	1.55	9.04	3.71	13.5	24.5
Red Poll	30	55	6915	37.4	3.81	1.42	9.09	3.40	5.98	7.45
British Friesian	30	65	8881	48.0	3.67	1.76	8.78	4.21	16.4	21.2
South Devon	30	105	7442	40.2	4.02	1.61	9.25	3.72	16.4	—
Lincoln red	25	65	7257	39.2	3.76	1.47	9.00	3.52	15.5	19.4
Dexter	60	80	4828	26.1	4.15	1.08	9.11	2.37	18.2	16.7
Kerry	30	55	5295	28.6	4.30	1.23	9.09	2.40	15.8	19.8
Ayrshire	35	95	7099	38.3	3.97	1.52	9.00	3.41	16.7	22.0

<sup>1</sup> Cp. Tocher, *loc. cit.* p. 55 *et seq.*

<sup>2</sup> Hincheliff, *Mitteilungen des Landwirtschaftlichen Institutes der Universität Leipzig*, 1904, 5, 1.

<sup>3</sup> Cp. Eccles and Shaw, *loc. cit.* *Bull.* 155, p. 76.

<sup>4</sup> *New York Agric. Exp. Station, Annual Reports*, 1890-1894.

The six central columns of the table give the total yield, average daily yield, average percentage and weight of fat and the average percentage and weight of solids-not-fat. From this table, it is seen that for the stage of the lactation period (15th to 200th day) under consideration, the order of the breeds from the point of view of yield is (1) British Friesian, (2) Dairy Shorthorn, (3) South Devon, (4) Lincoln Red Shorthorn, (5) Ayrshire, (6) Red Poll, (7) Jersey, (8) Guernsey, (9) Kerry, (10) Dexter, whilst the order for percentage of fat is (1) Jersey, (2) Guernsey, (3) Kerry, (4) Dexter, (5) South Devon, (6) Ayrshire, (7) Red Poll, (8) Dairy Shorthorn, (9) Lincoln Red Shorthorn, (10) British Friesian.

The position of the Jersey cows before the Guernsey cows for total yield may seem peculiar, but it is due to the fact that the records available for the lactation period considered show that the Jersey cows have maintained a relatively high yield of milk at the later stages of the lactation period. To show the decline in the yield of milk and the corresponding increase in the fat percentage, the last two columns in Table V show respectively the percentage decrease in yield and increase in fat content from the 100th to 200th day after calving.

In order to make ready comparison between the effects of the time since calving on the yield of the milk for each breed, the maximum yield

Table VI.

	Days' lactation									
	15	20	30	40	50	60	70	80	90	100
Jersey	90.5	93.5	97.1	99.5	100.0	99.7	98.3	96.2	94.4	93.5
Guernsey	93.2	96.6	100.0	98.4	97.8	94.3	92.6	90.3	88.6	87.4
Dairy Shorthorn	97.0	98.6	100.0	99.2	96.4	94.2	92.5	91.2	90.0	88.7
Red Poll	97.2	99.0	100.0	97.1	96.2	90.7	88.7	88.2	87.8	86.8
British Friesian	95.3	98.0	100.0	97.4	92.2	88.8	86.4	84.4	82.4	80.7
South Devon	97.8	99.4	100.0	98.1	93.6	87.9	83.4	80.1	76.0	73.4
Lincoln Red	99.2	100.0	99.2	95.8	92.4	90.3	88.2	86.6	84.8	83.1
Dexter	61.4	69.0	80.5	90.5	96.9	100.0	98.8	95.6	91.2	86.8
Kerry	96.2	98.4	100.0	98.9	94.5	88.4	84.6	81.2	78.5	76.8
Ayrshire	96.6	98.0	100.0	100.0	98.4	94.1	90.7	88.0	85.2	82.6

	Days' lactation									
	110	120	130	140	150	160	170	180	190	200
Jersey	93.2	92.6	92.0	91.4	90.8	90.3	89.7	89.1	88.5	88.2
Guernsey	84.5	82.3	81.1	80.0	78.8	77.6	76.5	76.0	75.3	74.8
Dairy Shorthorn	87.4	86.1	85.2	84.0	82.6	81.4	80.1	79.2	78.0	76.7
Red Poll	86.4	85.9	85.4	85.0	84.5	84.0	83.6	82.6	82.1	81.7
British Friesian	79.3	77.9	76.6	75.2	74.2	72.9	71.5	70.1	68.7	67.4
South Devon	71.9	70.3	69.2	68.1	67.0	66.2	—	—	—	—
Lincoln Red	81.9	80.6	78.9	77.6	76.4	74.6	73.4	71.7	—	—
Dexter	83.0	79.1	77.3	75.4	74.2	72.9	72.2	71.6	71.6	71.0
Kerry	75.2	73.5	71.9	71.4	69.6	68.6	67.4	67.0	65.8	64.7
Ayrshire	79.6	77.0	74.0	71.5	68.5	66.0	—	—	—	—

of milk has been represented as 100 and the variations from this figure with time are given in Table VI. The Jersey and Red Poll breeds show the minimum decrease in the yield as the lactation period advances, but comparisons in this respect especially at the later stages of lactation must be made with caution. Thus the influence of an uneven age distribution may be highly important during this period.

This matter is under consideration. In addition it has been shown by Cooke<sup>1</sup> that if cows are in calf the increase in the quality of the milk as it decreases in quantity is pronounced towards the end of the lactation period, but for farrow cows the shrinkage in quantity is accompanied by only a slight change in the quality.

Consequently a disturbing factor may exist which will preclude detailed comparison between the various breeds with regard to the changes in quality and quantity during the more advanced lactation periods.

The actual weight of fat yielded during the lactation period (0 to 200 days) remains moderately constant (Tables VII-XVII), but tends to rise towards the end of the lactation period under consideration. Mackintosh<sup>2</sup>, however, contends that the yield of fat diminishes and it must be observed that cows exhibited at a late stage of their lactation period probably yielded relatively exceptional quantities of milk. Here again the influence of the age of the cows may seriously affect the results, and a discussion of this matter will be made in a subsequent communication.

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Loc. cit.*

(Received November 6th, 1926.)

Table VII. *Milk of Jersey cows (1282 analyses).*

	Days since calving																					
	15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	
Morning milk:																						
Yield	(lb.)	16.2	16.6	17.2	17.6	17.8	17.7	17.4	17.1	16.9	16.8	16.7	16.6	16.5	16.4	16.3	16.2	16.1	16.0	15.9	15.8	15.8
Fat	(%)	4.74	4.58	4.36	4.32	4.36	4.44	4.52	4.60	4.68	4.76	4.84	4.92	5.00	5.08	5.16	5.24	5.32	5.40	5.48	5.57	5.65
Solids-not-fat	(%)	9.62	9.48	9.37	9.34	9.34	9.35	9.35	9.35	9.35	9.35	9.35	9.35	9.36	9.36	9.36	9.36	9.36	9.36	9.36	9.36	9.37
Evening milk:																						
Yield	(lb.)	14.2	14.8	15.4	15.8	15.9	15.8	15.6	15.2	14.8	14.6	14.6	14.5	14.4	14.3	14.2	14.1	14.0	13.9	13.8	13.8	13.8
Fat	(%)	5.62	5.48	5.34	5.30	5.31	5.34	5.38	5.44	5.49	5.54	5.60	5.66	5.71	5.76	5.82	5.87	5.92	5.98	6.03	6.09	6.14
Solids-not-fat	(%)	9.40	9.30	9.24	9.20	9.20	9.21	9.21	9.21	9.21	9.21	9.21	9.21	9.22	9.22	9.22	9.22	9.22	9.22	9.22	9.22	9.23
Day's milk:																						
Yield	(lb.)	30.4	31.4	32.6	33.4	33.7	33.5	33.0	32.3	31.7	31.4	31.3	31.1	30.9	30.7	30.5	30.3	30.1	29.9	29.7	29.6	29.6
Fat	(%)	5.16	5.00	4.82	4.79	4.82	4.84	4.94	5.02	5.07	5.13	5.20	5.27	5.34	5.38	5.44	5.54	5.62	5.66	5.73	5.81	5.85
Solids-not-fat	(%)	9.55	9.40	9.31	9.27	9.27	9.28	9.28	9.28	9.29	9.29	9.29	9.29	9.29	9.29	9.29	9.29	9.29	9.29	9.29	9.29	9.30
Wt. of fat (lb.):																						
Morning milk		0.77	0.76	0.75	0.76	0.78	0.79	0.79	0.79	0.79	0.80	0.81	0.82	0.82	0.83	0.84	0.85	0.86	0.86	0.87	0.88	0.89
Evening "		0.80	0.81	0.82	0.84	0.84	0.83	0.84	0.83	0.82	0.81	0.82	0.82	0.82	0.82	0.82	0.83	0.83	0.83	0.83	0.84	0.84
Day's "		1.57	1.57	1.57	1.60	1.62	1.62	1.62	1.62	1.61	1.61	1.63	1.64	1.65	1.65	1.66	1.66	1.69	1.69	1.70	1.72	1.73
Wt. of solids-not-fat (lb.):																						
Morning milk		1.56	1.57	1.61	1.64	1.65	1.65	1.63	1.60	1.58	1.57	1.56	1.55	1.54	1.53	1.52	1.51	1.50	1.49	1.48	1.49	
Evening "		1.34	1.38	1.42	1.45	1.45	1.45	1.40	1.36	1.34	1.34	1.34	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1.27	1.27	
Day's "		2.90	2.95	3.03	3.09	3.10	3.10	3.08	3.00	2.94	2.91	2.90	2.89	2.87	2.85	2.83	2.82	2.80	2.78	2.76	2.75	2.76
Wt. of milk containing 1 lb. of fat																						
		19.4	20.0	20.7	20.9	20.8	20.7	20.3	20.2	19.7	19.4	19.2	18.9	18.7	18.6	18.4	18.1	17.8	17.7	17.5	17.2	17.1
Wt. of total solids (lb.)																						
		4.47	4.52	4.60	4.69	4.72	4.72	4.71	4.62	4.55	4.52	4.53	4.52	4.52	4.52	4.49	4.50	4.49	4.47	4.46	4.47	4.49
Percentage of fat in total solids																						
		35.1	34.7	34.3	34.1	34.3	34.3	34.3	35.0	35.4	35.7	36.0	36.3	36.5	36.6	36.9	37.3	37.6	37.8	38.1	38.4	38.6

Table VIII. *Milk of Guernsey cows (582 analyses).*

		Days since calving																				
		15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210
Morning milk:																						
Yield	(lb.)	17.7	18.3	18.9	18.6	18.5	17.9	17.6	17.2	16.9	16.7	16.2	15.8	15.6	15.4	15.2	15.0	14.8	14.7	14.6	14.5	14.4
Fat	(%)	4.46	4.30	4.16	4.16	4.19	4.25	4.32	4.38	4.45	4.52	4.60	4.66	4.73	4.80	4.88	4.96	5.04	5.13	5.22	5.30	5.39
Solids-not-fat	(%)	9.42	9.36	9.30	9.27	9.25	9.25	9.25	9.25	9.26	9.28	9.29	9.31	9.32	9.34	9.36	9.38	9.41	9.43	9.45	9.49	9.53
Evening milk:																						
Yield	(lb.)	14.7	15.3	15.9	15.6	15.5	14.9	14.6	14.2	13.9	13.7	13.2	12.8	12.6	12.4	12.2	12.0	11.8	11.7	11.6	11.5	11.4
Fat	(%)	5.07	5.01	4.92	4.92	4.93	4.94	4.95	5.01	5.09	5.11	5.16	5.24	5.29	5.34	5.40	5.46	5.53	5.60	5.67	5.75	5.83
Solids-not-fat	(%)	9.31	9.25	9.19	9.16	9.14	9.14	9.14	9.14	9.15	9.17	9.18	9.20	9.21	9.23	9.25	9.27	9.30	9.32	9.34	9.38	9.42
Day's milk:																						
Yield	(lb.)	32.4	33.6	34.8	34.2	34.0	32.8	32.2	31.4	30.8	30.4	29.4	28.6	28.2	27.8	27.4	27.0	26.6	26.4	26.2	26.0	25.8
Fat	(%)	4.75	4.70	4.48	4.48	4.50	4.56	4.59	4.65	4.74	4.77	4.86	4.93	5.00	5.04	5.10	5.18	5.26	5.37	5.42	5.50	5.61
Solids-not-fat	(%)	9.38	9.31	9.25	9.22	9.21	9.21	9.21	9.21	9.22	9.24	9.25	9.26	9.27	9.29	9.31	9.33	9.36	9.39	9.40	9.44	9.48
Wt. of fat (lb.):																						
Morning milk		0.79	0.81	0.78	0.78	0.78	0.76	0.76	0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.74	0.75	0.76	0.76	0.77	0.78
Evening "		0.75	0.77	0.78	0.77	0.77	0.74	0.72	0.71	0.71	0.70	0.68	0.67	0.67	0.66	0.66	0.66	0.65	0.66	0.66	0.66	0.65
Day's "		1.54	1.58	1.56	1.55	1.55	1.50	1.48	1.46	1.46	1.45	1.43	1.41	1.41	1.40	1.40	1.40	1.40	1.42	1.42	1.43	1.45
Wt. of solids-not-fat (lb.):																						
Morning milk		1.67	1.71	1.76	1.72	1.71	1.66	1.63	1.59	1.57	1.55	1.51	1.47	1.45	1.44	1.42	1.41	1.40	1.39	1.38	1.37	1.37
Evening "		1.47	1.42	1.46	1.43	1.42	1.36	1.34	1.30	1.27	1.26	1.21	1.18	1.16	1.15	1.13	1.11	1.10	1.09	1.08	1.08	1.07
Day's "		3.04	3.13	3.22	3.15	3.13	3.12	2.97	2.89	2.84	2.81	2.72	2.65	2.61	2.59	2.55	2.52	2.50	2.48	2.48	2.45	2.44
Wt. of milk containing 1 lb. of fat		21.0	21.2	22.3	22.0	21.9	21.8	21.7	21.5	21.1	21.0	20.6	20.3	20.0	19.8	19.5	19.3	19.0	18.6	18.4	18.2	17.8
Wt. of total solids (lb.)		4.58	4.71	4.78	4.70	4.68	4.52	4.45	4.35	4.30	4.26	4.15	4.06	4.02	3.99	3.95	3.92	3.90	3.88	3.88	3.88	3.89
Percentage of fat in total solids		33.6	33.5	32.6	33.0	33.1	33.2	33.3	33.6	34.0	34.1	34.5	34.7	35.1	35.1	35.5	35.7	35.9	36.5	36.6	36.9	37.3

Table IX. *Milk of Dairy Shorthorn cows (2228 analyses).*

		Days since calving																			
		15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
Morning milk:																					
Yield	(lb.)	24.0	24.3	24.6	24.4	23.7	23.1	22.7	22.4	22.1	21.8	21.5	21.2	21.0	20.7	20.4	20.1	19.8	19.6	19.3	19.0
Fat	(%)	3.84	3.76	3.62	3.50	3.42	3.35	3.32	3.30	3.29	3.32	3.38	3.44	3.52	3.59	3.68	3.75	3.88	3.99	4.10	4.20
Solids-not-fat	(%)	9.32	9.23	9.11	9.04	9.01	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.02	9.03	9.06	9.12	9.17	9.22	9.27	9.32
Evening milk:																					
Yield	(lb.)	21.2	21.6	22.0	21.8	21.2	20.8	20.4	20.1	19.8	19.5	19.2	18.9	18.7	18.4	18.1	17.8	17.5	17.3	17.0	16.7
Fat	(%)	4.40	4.31	4.16	4.04	3.92	3.83	3.76	3.72	3.71	3.73	3.78	3.85	3.92	4.00	4.08	4.17	4.26	4.36	4.45	4.55
Solids-not-fat	(%)	9.20	9.11	9.02	8.96	8.92	8.92	8.92	8.92	8.92	8.92	8.92	8.92	8.93	8.95	8.98	9.03	9.07	9.12	9.17	9.22
Day's milk:																					
Yield	(lb.)	45.2	45.9	46.6	46.2	44.9	43.9	43.1	42.5	41.9	41.3	40.7	40.1	39.7	39.1	38.5	37.9	37.3	36.9	36.3	35.7
Fat	(%)	4.10	4.02	3.81	3.76	3.66	3.59	3.53	3.50	3.49	3.51	3.59	3.64	3.70	3.76	3.87	3.93	4.07	4.18	4.27	4.37
Solids-not-fat	(%)	9.26	9.16	9.06	9.02	8.98	8.97	8.97	8.97	8.97	8.97	8.97	8.97	8.98	9.00	9.03	9.10	9.14	9.19	9.23	9.28
Wt. of fat (lb.):																					
Morning milk		0.92	0.91	0.86	0.86	0.81	0.78	0.76	0.74	0.73	0.72	0.73	0.73	0.73	0.74	0.74	0.75	0.77	0.78	0.79	0.80
Evening "		0.93	0.93	0.92	0.88	0.83	0.80	0.77	0.75	0.74	0.74	0.73	0.73	0.73	0.73	0.74	0.74	0.75	0.76	0.76	0.76
Day's "		1.86	1.84	1.78	1.74	1.64	1.57	1.52	1.49	1.47	1.45	1.46	1.46	1.47	1.47	1.49	1.49	1.52	1.54	1.55	1.56
Wt. of solids-not-fat (lb.):																					
Morning milk		2.24	2.25	2.24	2.21	2.14	2.08	2.04	2.02	1.99	1.96	1.94	1.91	1.89	1.87	1.85	1.83	1.82	1.81	1.79	1.77
Evening "		1.95	1.97	1.99	1.96	1.89	1.86	1.82	1.79	1.77	1.74	1.71	1.68	1.67	1.64	1.62	1.61	1.59	1.58	1.56	1.54
Day's "		4.19	4.22	4.23	4.17	4.03	3.94	3.86	3.81	3.76	3.70	3.65	3.59	3.56	3.51	3.47	3.44	3.41	3.39	3.35	3.31
Wt. of milk containing 1 lb. of fat		24.3	24.9	26.2	26.5	27.4	28.0	28.4	28.5	28.5	28.5	27.9	27.5	27.0	26.6	25.8	25.4	24.2	24.0	23.4	22.9
Wt. of total solids (lb.)		6.05	6.06	6.01	5.91	5.67	5.51	5.38	5.30	5.23	5.15	5.11	5.05	5.03	4.98	4.96	4.93	4.93	4.93	4.90	4.87
Percentage of fat in total solids		30.4	29.6	29.0	28.5	28.3	28.1	28.1	28.1	28.2	28.6	28.9	29.2	29.5	30.0	30.2	30.8	31.2	31.2	31.6	32.0

Table X. *Milk of Red Poll cows (748 analyses).*

		Days since calving																				
		15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210
<b>Morning milk:</b>																						
Yield	(lb.)	21.8	22.2	22.4	21.8	21.0	20.4	20.0	19.9	19.8	19.6	19.5	19.4	19.3	19.2	19.1	19.0	18.9	18.7	18.6	18.5	18.4
Fat	(%)	3.83	3.74	3.63	3.56	3.52	3.53	3.53	3.53	3.54	3.56	3.58	3.60	3.62	3.64	3.66	3.70	3.74	3.78	3.82	3.88	3.94
Solids-not-fat	(%)	9.44	9.30	9.16	9.10	9.08	9.08	9.08	9.09	9.09	9.09	9.09	9.10	9.10	9.10	9.10	9.10	9.10	9.11	9.12	9.12	9.12
<b>Evening milk:</b>																						
Yield	(lb.)	19.4	19.8	20.0	19.4	18.6	18.0	17.6	17.5	17.4	17.2	17.1	17.0	16.9	16.8	16.7	16.6	16.5	16.3	16.2	16.1	16.0
Fat	(%)	4.26	4.19	4.06	3.96	3.92	3.92	3.93	3.94	3.95	3.97	3.99	4.01	4.02	4.04	4.06	4.09	4.12	4.14	4.18	4.23	4.28
Solids-not-fat	(%)	9.36	9.22	9.08	9.02	9.00	9.00	9.00	9.01	9.01	9.01	9.01	9.02	9.02	9.02	9.02	9.02	9.03	9.03	9.04	9.04	9.04
<b>Day's milk:</b>																						
Yield	(lb.)	41.2	42.0	42.4	41.2	39.6	38.4	37.6	37.4	37.2	36.8	36.6	36.4	36.2	36.0	35.8	35.6	35.4	35.0	34.8	34.6	34.4
Fat	(%)	4.04	3.95	3.83	3.76	3.71	3.71	3.72	3.73	3.74	3.76	3.77	3.79	3.80	3.82	3.84	3.89	3.91	3.95	3.97	4.04	4.10
Solids-not-fat	(%)	9.41	9.27	9.14	9.07	9.05	9.05	9.06	9.06	9.06	9.06	9.06	9.07	9.07	9.07	9.07	9.07	9.08	9.08	9.09	9.09	9.09
<b>Wt. of fat (lb.):</b>																						
Morning milk		0.84	0.83	0.81	0.78	0.74	0.72	0.71	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.71	0.71	0.72	0.73
Evening "		0.83	0.83	0.81	0.77	0.73	0.71	0.69	0.69	0.69	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.69
Day's "		1.66	1.66	1.62	1.54	1.47	1.42	1.40	1.39	1.39	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.38	1.40	1.41
<b>Wt. of solids-not-fat (lb.):</b>																						
Morning milk		2.06	2.07	2.06	1.99	1.91	1.85	1.82	1.81	1.80	1.78	1.77	1.77	1.76	1.75	1.74	1.73	1.72	1.71	1.70	1.69	1.68
Evening "		1.82	1.83	1.84	1.75	1.68	1.62	1.59	1.58	1.57	1.56	1.54	1.53	1.53	1.52	1.51	1.50	1.47	1.47	1.46	1.45	
Day's "		3.88	3.89	3.89	3.74	3.59	3.47	3.40	3.39	3.37	3.33	3.31	3.30	3.28	3.27	3.25	3.23	3.22	3.18	3.16	3.14	3.12
<b>Wt. of milk containing 1 lb. of fat (lb.):</b>																						
		24.8	25.3	26.2	26.7	26.9	27.0	26.8	26.9	26.8	26.7	26.5	26.4	26.2	26.1	25.9	25.8	25.5	25.4	25.2	24.7	23.4
<b>Wt. of total solids (lb.):</b>																						
		5.54	5.55	5.51	5.28	5.06	4.89	4.80	4.78	4.76	4.71	4.69	4.68	4.66	4.65	4.63	4.61	4.60	4.56	4.54	4.54	4.53
<b>Percentage of fat in total solids</b>																						
		30.0	29.9	29.4	29.2	29.1	29.2	29.2	29.2	29.1	29.3	29.4	29.5	29.6	29.7	29.8	29.9	30.0	30.3	30.3	30.8	31.2

Table XI. *Milk of British Friesian cows (374 analyses).*

		Days since calving																			
		15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200
<b>Morning milk:</b>																					
Yield	(lb.)	30.0	30.8	31.4	30.6	29.1	28.1	27.4	26.8	26.2	25.7	25.3	24.9	24.5	24.1	23.8	23.4	23.0	22.6	22.2	21.8
Fat	(%)	3.68	3.58	3.42	3.28	3.18	3.12	3.12	3.12	3.16	3.22	3.30	3.38	3.46	3.53	3.60	3.68	3.74	3.84	3.92	3.99
Solids-not-fat	(%)	9.12	9.04	8.92	8.84	8.79	8.78	8.78	8.78	8.78	8.78	8.78	8.78	8.78	8.78	8.78	8.79	8.80	8.82	8.84	8.85
<b>Evening milk:</b>																					
Yield	(lb.)	26.0	26.8	27.4	26.6	25.1	24.1	23.4	22.8	22.2	21.7	21.3	20.9	20.5	20.1	19.8	19.4	19.0	18.6	18.2	17.8
Fat	(%)	4.10	4.02	3.85	3.72	3.62	3.56	3.56	3.59	3.63	3.69	3.76	3.82	3.89	3.96	4.03	4.08	4.16	4.22	4.29	4.36
Solids-not-fat	(%)	9.01	8.93	8.81	8.73	8.68	8.67	8.67	8.67	8.67	8.67	8.67	8.67	8.67	8.67	8.67	8.68	8.69	8.71	8.73	8.76
<b>Day's milk:</b>																					
Yield	(lb.)	56.0	57.6	58.8	57.2	54.2	52.2	50.8	49.6	48.4	47.4	46.6	45.8	45.0	44.2	43.6	42.8	42.0	41.2	40.4	39.6
Fat	(%)	3.88	3.78	3.62	3.50	3.39	3.34	3.34	3.36	3.43	3.48	3.56	3.62	3.69	3.79	3.86	3.90	3.90	4.00	4.06	4.13
Solids-not-fat	(%)	9.08	9.00	8.87	8.79	8.75	8.74	8.74	8.74	8.74	8.74	8.74	8.74	8.74	8.74	8.74	8.75	8.76	8.78	8.80	8.81
<b>Wt. of fat (lb.):</b>																					
Morning milk		1.10	1.10	1.07	1.01	0.93	0.88	0.86	0.85	0.85	0.85	0.86	0.86	0.86	0.86	0.87	0.88	0.88	0.89	0.89	0.89
Evening "		1.07	1.06	1.06	0.99	0.91	0.86	0.83	0.82	0.81	0.80	0.80	0.80	0.80	0.80	0.80	0.79	0.79	0.78	0.78	0.78
Days "		2.17	2.18	2.13	2.00	1.84	1.74	1.69	1.67	1.66	1.65	1.66	1.66	1.66	1.67	1.68	1.67	1.67	1.67	1.67	1.67
<b>Wt. of solids-not-fat (lb.):</b>																					
Morning milk		2.74	2.79	2.80	2.71	2.56	2.47	2.41	2.35	2.30	2.28	2.22	2.19	2.16	2.12	2.09	2.06	2.02	2.00	1.97	1.94
Evening "		2.34	2.39	2.42	2.32	2.18	2.08	2.03	1.98	1.93	1.88	1.84	1.82	1.78	1.75	1.72	1.69	1.66	1.63	1.59	1.56
Days "		5.08	5.18	5.22	5.03	4.74	4.56	4.44	4.33	4.23	4.14	4.07	4.01	3.94	3.87	3.87	3.75	3.68	3.63	3.56	3.50
Wt. of milk containing 1 lb. of fat		25.8	26.4	27.6	28.6	29.4	30.0	30.0	29.7	29.2	28.7	28.0	27.6	27.1	26.5	26.0	25.6	25.0	24.7	24.2	23.7
Wt. of total solids		7.25	7.36	7.35	7.03	6.58	6.30	6.13	6.00	5.89	5.79	5.73	5.67	5.60	5.54	5.49	5.42	5.36	5.30	5.23	5.17
Percentage of fat in total solids		29.9	29.6	29.0	28.5	28.0	27.6	27.6	27.9	28.2	28.5	29.0	29.3	29.7	30.2	30.6	30.8	31.4	31.5	32.0	32.3



Table XII. *Milk of Devon cows (78 analyses).*

	Days since calving							
	15	20	30	40	50	60	70	80
Morning milk:								
Yield (lb.)	24.0	24.5	25.3	25.6	25.6	25.5	25.1	24.8
Fat (%)	4.60	4.49	4.32	4.17	4.04	3.96	3.92	3.90
Solids-not-fat (,,)	9.42	9.36	9.27	9.22	9.16	9.13	9.10	9.08
Evening milk:								
Yield (lb.)	20.0	20.5	21.3	21.6	21.6	21.5	21.1	20.8
Fat (%)	4.88	4.83	4.70	4.57	4.47	4.40	4.37	4.35
Solids-not-fat (,,)	9.30	9.24	9.15	9.10	9.04	9.01	8.98	8.96
Day's milk:								
Yield (lb.)	44.0	45.0	46.6	47.2	47.2	47.0	46.2	45.6
Fat (%)	4.73	4.64	4.48	4.30	4.24	4.17	4.14	4.12
Solids-not-fat (,,)	9.37	9.30	9.23	9.19	9.14	9.07	9.05	9.03
Weight of fat (lb.):								
Morning milk	1.10	1.10	1.09	1.06	1.03	1.01	0.99	0.97
Evening „	0.98	0.99	1.00	0.97	0.97	0.95	0.92	0.91
Day's „	2.08	2.09	2.09	2.03	2.00	1.96	1.91	1.88
Wt. of solids-not-fat (lb.):								
Morning milk	2.26	2.29	2.35	2.36	2.35	2.32	2.28	2.26
Evening „	1.86	1.89	1.95	1.97	1.96	1.94	1.89	1.86
Day's „	4.12	4.18	4.30	4.33	4.31	4.26	4.17	4.12
Wt. of milk containing 1 lb. of fat	21.1	21.5	22.3	23.3	23.6	24.0	24.2	24.2
Wt. of total solids (lb.)	6.20	6.27	6.39	6.34	6.31	6.22	6.08	6.00
Percentage of fat in total solids	33.5	33.4	32.7	32.0	31.7	31.5	31.4	31.4

Table XIII. *Milk of South Devon cows (110 analyses).*

		Days since calving															
		15	20	30	40	50	60	70	80	90	100	110	120	130	140		150
Morning milk:																	
Yield	(lb.)	27.0	27.4	27.6	27.1	25.9	24.4	23.2	22.2	21.3	20.6	20.2	19.8	19.5	19.2	18.9	18.7
Fat	(%)	4.14	4.09	3.98	3.90	3.83	3.78	3.75	3.72	3.70	3.69	3.69	3.70	3.70	3.71	3.71	3.73
Solids-not-fat	(%)	9.51	9.48	9.43	9.39	9.35	9.32	9.30	9.28	9.27	9.26	9.26	9.26	9.26	9.26	9.26	9.26
Evening milk:																	
Yield	(lb.)	24.4	24.8	25.0	24.5	23.3	21.8	20.6	19.6	18.7	18.0	17.6	17.2	16.9	16.6	16.3	16.1
Fat	(%)	4.68	4.62	4.50	4.42	4.37	4.32	4.28	4.25	4.22	4.21	4.21	4.21	4.21	4.22	4.22	4.23
Solids-not-fat	(%)	9.37	9.34	9.29	9.25	9.21	9.18	9.16	9.14	9.13	9.12	9.12	9.12	9.12	9.12	9.12	9.12
Day's milk:																	
Yield	(lb.)	51.4	52.2	52.6	51.6	49.2	46.2	43.8	41.8	40.0	38.6	37.8	37.0	36.4	35.8	35.2	34.8
Fat	(%)	4.39	4.33	4.21	4.13	4.10	4.04	3.99	3.97	3.95	3.94	3.94	3.95	3.95	3.96	3.96	3.97
Solids-not-fat	(%)	9.45	9.41	9.38	9.33	9.29	9.25	9.23	9.22	9.21	9.20	9.20	9.20	9.20	9.20	9.20	9.20
Wt. of fat (lb.):																	
Morning milk		1.12	1.12	1.10	1.06	0.99	0.92	0.87	0.83	0.79	0.76	0.75	0.73	0.72	0.71	0.70	0.70
Evening "		1.14	1.15	1.13	1.06	1.02	0.94	0.88	0.83	0.79	0.76	0.74	0.72	0.71	0.70	0.69	0.68
Day's "		2.26	2.27	2.23	2.14	2.01	1.86	1.75	1.66	1.58	1.52	1.49	1.45	1.43	1.41	1.39	1.38
Wt. of solids-not-fat (lb.):																	
Morning milk		2.57	2.60	2.60	2.54	2.42	2.28	2.16	2.06	1.98	1.91	1.89	1.84	1.80	1.78	1.75	1.73
Evening "		2.29	2.32	2.32	2.27	2.15	2.00	1.89	1.79	1.70	1.64	1.60	1.57	1.56	1.53	1.50	1.48
Day's "		4.86	4.92	4.92	4.81	4.57	4.28	4.05	3.86	3.68	3.55	3.49	3.41	3.36	3.31	3.25	3.21
Wt. of milk containing 1 lb. of fat (lb.):		22.7	23.0	23.5	24.1	24.5	24.8	25.0	25.2	25.4	25.4	25.4	25.5	25.4	25.4	25.3	25.2
Wt. of total solids (lb.):		7.12	7.19	7.15	6.95	6.58	6.14	5.80	5.52	5.26	5.07	4.98	4.86	4.79	4.72	4.64	4.59
Percentage of fat in total solids		31.7	31.5	31.1	30.8	30.6	30.4	30.2	30.1	30.0	30.0	29.9	29.9	29.9	29.9	30.0	30.1

Table XIV. *Milk of Lincoln Red cows (378 analyses).*

	Days since calving																	
	15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180
Morning milk:																		
Yield	(lb.) 24.8	25.0	24.8	24.0	23.2	22.7	22.2	21.8	21.4	21.0	20.7	20.4	20.0	19.7	19.4	19.0	18.7	18.3
Fat	(%) 3.70	3.61	3.46	3.25	3.27	3.22	3.22	3.26	3.31	3.38	3.45	3.53	3.61	3.68	3.75	3.81	3.88	3.95
Solids-not-fat	(„) 9.26	9.22	9.15	9.09	9.05	9.02	8.99	8.98	8.98	8.98	8.98	8.98	8.98	8.98	8.98	8.98	9.01	9.06
Evening milk:																		
Yield	(lb.) 22.0	22.2	22.0	21.2	20.4	19.9	19.4	19.0	18.6	18.2	17.9	17.6	17.2	16.9	16.6	16.2	15.9	15.5
Fat	(%) 4.12	4.05	3.91	3.81	3.74	3.71	3.71	3.74	3.78	3.84	3.90	3.97	4.04	4.09	4.15	4.21	4.28	4.35
Solids-not-fat	(„) 9.19	9.15	9.08	9.02	8.98	8.95	8.92	8.91	8.91	8.91	8.91	8.91	8.91	8.91	8.91	8.91	8.94	8.99
Day's milk:																		
Yield	(lb.) 46.8	47.2	46.8	45.2	43.6	42.6	41.6	40.8	40.0	39.2	38.6	38.0	37.2	36.6	36.0	35.2	34.6	33.8
Fat	(%) 3.87	3.81	3.68	3.58	3.48	3.45	3.45	3.47	3.52	3.59	3.62	3.74	3.82	3.88	3.94	3.98	4.08	4.16
Solids-not-fat	(„) 9.23	9.19	9.11	9.06	9.02	8.99	8.96	8.95	8.95	8.95	8.95	8.95	8.95	8.95	8.95	8.95	8.98	9.03
Wt. of fat (lb.):																		
Morning milk	0.90	0.90	0.86	0.81	0.76	0.74	0.72	0.71	0.71	0.71	0.71	0.72	0.72	0.73	0.73	0.72	0.73	0.73
Evening „	0.91	0.90	0.86	0.81	0.76	0.74	0.72	0.71	0.70	0.70	0.68	0.70	0.70	0.69	0.69	0.68	0.68	0.68
Day's „	1.81	1.80	1.72	1.62	1.52	1.48	1.44	1.42	1.41	1.41	1.39	1.42	1.42	1.42	1.42	1.40	1.41	1.41
Wt. of solids-not-fat (lb.):																		
Morning milk	2.30	2.30	2.27	2.18	2.10	2.05	2.00	1.96	1.93	1.88	1.86	1.83	1.80	1.77	1.74	1.71	1.69	1.66
Evening „	2.02	2.03	2.00	1.91	1.83	1.78	1.73	1.70	1.66	1.62	1.55	1.57	1.53	1.51	1.48	1.45	1.42	1.39
Day's „	4.32	4.33	4.27	4.09	3.93	3.83	3.73	3.66	3.59	3.50	3.41	3.40	3.33	3.28	3.22	3.16	3.11	3.05
Wt. of milk containing 1 lb. of fat (lb.)																		
	25.8	26.2	27.2	27.9	28.6	28.6	28.9	28.7	28.4	27.8	26.7	26.2	25.8	25.3	25.1	24.5	24.0	
Wt. of total solids (lb.):																		
	6.13	6.13	5.99	5.71	5.45	5.31	5.17	5.08	5.00	4.91	4.80	4.82	4.75	4.70	4.64	4.56	4.52	4.46
Percentage of fat in total solids																		
	29.5	29.4	28.8	28.4	27.9	27.9	27.9	28.0	28.2	28.7	29.0	29.5	29.9	30.3	30.6	30.7	31.2	31.6

Table XV. *Milk of Dexter cows (160 analyses).*

		Days since calving																						
		15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210		
<b>Morning milk:</b>																								
Yield	(lb.)	10.6	11.8	13.6	15.2	16.2	16.7	16.5	16.0	15.3	14.6	14.0	13.4	13.1	12.8	12.6	12.4	12.3	12.2	12.2	12.1	12.1		
Fat	(%)	4.72	4.53	4.22	3.96	3.80	3.72	3.70	3.70	3.72	3.74	3.77	3.81	3.87	3.92	3.99	4.06	4.16	4.25	4.33	4.44	4.53		
Solids-not-fat	(%)	9.80	9.63	9.39	9.24	9.14	9.09	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06		
<b>Evening milk:</b>																								
Yield	(lb.)	8.8	10.0	11.8	13.4	14.4	14.9	14.7	14.2	13.5	12.8	12.2	11.6	11.3	11.0	10.8	10.6	10.5	10.4	10.4	10.3	10.3		
Fat	(%)	5.20	5.00	4.69	4.43	4.25	4.14	4.11	4.11	4.12	4.13	4.15	4.18	4.23	4.28	4.34	4.41	4.48	4.56	4.66	4.74	4.84		
Solids-not-fat	(%)	9.74	9.57	9.33	9.18	9.08	9.03	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00		
<b>Day's milk:</b>																								
Yield	(lb.)	19.4	21.8	25.4	28.6	30.6	31.6	31.2	30.2	28.8	27.4	26.2	25.0	24.4	23.8	23.4	23.0	22.8	22.6	22.6	22.4	22.4		
Fat	(%)	5.00	4.77	4.41	4.16	4.00	3.92	3.90	3.84	3.92	3.94	3.97	4.00	4.06	4.08	4.15	4.22	4.30	4.40	4.51	4.60	4.69		
Solids-not-fat	(%)	9.78	9.61	9.37	9.22	9.12	9.07	9.04	9.04	9.04	9.04	9.04	9.04	9.04	9.04	9.04	9.04	9.04	9.04	9.04	9.04	9.04		
<b>Wt. of fat (lb.):</b>																								
Morning milk		0.50	0.54	0.57	0.60	0.61	0.62	0.61	0.58	0.57	0.55	0.53	0.51	0.50	0.50	0.50	0.50	0.51	0.52	0.53	0.54	0.55		
Evening "		0.46	0.50	0.55	0.59	0.61	0.62	0.61	0.58	0.56	0.53	0.51	0.49	0.48	0.47	0.47	0.47	0.48	0.49	0.49	0.49	0.50		
Day's "		0.97	1.04	1.12	1.19	1.22	1.24	1.22	1.16	1.13	1.08	1.04	1.00	0.99	0.97	0.97	0.97	0.98	1.00	1.02	1.03	1.05		
<b>Wt. of solids-not-fat (lb.):</b>																								
Morning milk		1.04	1.14	1.28	1.41	1.48	1.52	1.50	1.45	1.39	1.32	1.27	1.22	1.19	1.16	1.14	1.12	1.11	1.11	1.11	1.10	1.09		
Evening "		0.86	0.96	1.10	1.23	1.31	1.35	1.32	1.28	1.22	1.15	1.10	1.04	1.02	0.99	0.97	0.95	0.95	0.94	0.94	0.93	0.93		
Day's "		1.90	2.10	2.38	2.64	2.79	2.87	2.82	2.73	2.61	2.47	2.37	2.26	2.21	2.15	2.11	2.07	2.06	2.05	2.05	2.03	2.02		
<b>Wt. of milk containing 1 lb. of fat (lb.):</b>																								
		20.0	21.0	22.7	24.0	25.1	25.5	25.6	26.0	25.5	25.4	25.2	25.0	24.6	24.5	24.1	23.7	23.3	22.6	22.2	21.7	21.4		
<b>Wt. of total solids (lb.):</b>																								
		2.87	3.14	3.50	3.83	4.01	4.11	4.04	3.89	3.74	3.55	3.41	3.26	3.20	3.12	3.06	3.04	3.04	3.05	3.07	3.06	3.07		
<b>Percentage of fat in total solids</b>																								
		33.8	33.1	32.0	31.1	30.4	30.2	30.1	29.8	30.2	30.4	30.5	30.7	30.9	31.1	31.5	31.9	32.2	32.8	33.2	33.7	34.2		

Table XVI. *Milk of Kerry cows (348 analyses).*

		Days since calving																				
		15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210
<b>Morning milk:</b>																						
Yield	(lb.)	18.3	18.7	19.0	18.8	18.0	16.9	16.2	15.6	15.1	14.8	14.5	14.2	13.9	13.8	13.5	13.3	13.1	13.0	12.8	12.6	12.4
Fat	(%)	4.33	4.16	3.91	3.78	3.74	3.74	3.75	3.77	3.80	3.84	3.89	3.94	4.00	4.08	4.18	4.28	4.40	4.50	4.64	4.80	4.97
Solids-not-fat	(%)	9.33	9.28	9.20	9.15	9.12	9.11	9.10	9.10	9.10	9.10	9.10	9.10	9.10	9.10	9.10	9.11	9.12	9.13	9.15	9.20	9.25
<b>Evening milk:</b>																						
Yield	(lb.)	16.5	16.9	17.2	17.0	16.2	15.1	14.4	13.8	13.3	13.0	12.7	12.4	12.1	12.0	11.7	11.5	11.3	11.2	11.0	10.8	10.6
Fat	(%)	4.74	4.64	4.50	4.42	4.39	4.39	4.40	4.42	4.46	4.49	4.53	4.58	4.63	4.69	4.75	4.82	4.90	5.01	5.11	5.22	5.34
Solids-not-fat	(%)	9.23	9.18	9.10	9.05	9.02	9.01	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.01	9.02	9.03	9.05	9.10
<b>Day's milk:</b>																						
Yield	(lb.)	34.8	35.6	36.2	35.8	34.2	32.0	30.6	29.4	28.4	27.8	27.2	26.6	26.0	25.8	25.2	24.8	24.4	24.2	23.8	23.4	23.0
Fat	(%)	4.50	4.38	4.17	4.06	4.03	4.03	4.05	4.07	4.08	4.14	4.20	4.25	4.31	4.38	4.49	4.52	4.63	4.71	4.83	4.96	5.17
Solids-not-fat	(%)	9.29	9.24	9.16	9.11	9.07	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.07	9.08	9.09	9.11	9.15	9.21
<b>Wt. of fat (lb.):</b>																						
Morning milk		0.79	0.78	0.74	0.71	0.67	0.63	0.61	0.59	0.57	0.56	0.56	0.56	0.56	0.56	0.57	0.57	0.58	0.58	0.59	0.60	0.62
Evening "		0.78	0.78	0.77	0.75	0.71	0.66	0.63	0.61	0.59	0.58	0.58	0.57	0.56	0.56	0.56	0.55	0.55	0.56	0.56	0.56	0.57
Day's "		1.57	1.56	1.51	1.46	1.38	1.29	1.24	1.20	1.16	1.15	1.14	1.13	1.12	1.12	1.13	1.12	1.13	1.14	1.15	1.16	1.19
<b>Wt. of solids-not-fat (lb.):</b>																						
Morning milk		1.71	1.74	1.75	1.72	1.64	1.54	1.47	1.42	1.38	1.35	1.32	1.29	1.27	1.25	1.23	1.21	1.20	1.19	1.17	1.16	1.15
Evening "		1.52	1.55	1.57	1.54	1.46	1.36	1.30	1.24	1.20	1.17	1.14	1.12	1.09	1.08	1.05	1.04	1.02	1.01	1.00	0.98	0.97
Day's "		3.23	3.29	3.32	3.26	3.10	2.90	2.77	2.66	2.58	2.52	2.46	2.41	2.36	2.33	2.28	2.25	2.22	2.20	2.17	2.14	2.12
<b>Wt. of milk containing 1 lb. of fat (lb.):</b>																						
Wt. of total solids (lb.)		4.80	4.85	4.83	4.72	4.48	4.19	4.01	3.86	3.74	3.67	3.60	3.54	3.48	3.45	3.41	3.37	3.35	3.34	3.32	3.30	3.31
Percentage of fat in total solids		32.7	32.2	31.3	31.0	30.8	30.6	30.9	31.1	31.1	31.3	31.7	31.9	32.2	32.5	33.1	33.3	33.7	34.2	34.6	35.2	36.0

Table XVII. *Milk of Ayrshire cows (284 analyses).*

		Days since calving															
		15	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160
<b>Morning milk:</b>																	
Yield	(lb.)	24.2	24.5	25.0	25.0	24.6	23.6	22.8	22.2	21.5	20.9	20.2	19.6	18.9	18.3	17.6	17.0
Fat	(%)	4.26	4.18	3.97	3.82	3.68	3.56	3.45	3.38	3.36	3.37	3.39	3.45	3.53	3.64	3.77	3.89
Solids-not-fat	(%)	9.41	9.31	9.14	9.04	8.98	8.94	8.94	8.94	8.95	8.96	8.96	8.98	8.99	9.02	9.04	9.07
<b>Evening milk:</b>																	
Yield	(lb.)	21.1	21.4	21.9	21.9	21.5	20.5	19.7	19.1	18.4	17.8	17.1	16.5	15.8	15.2	14.5	13.9
Fat	(%)	4.73	4.65	4.50	4.37	4.26	4.15	4.06	3.99	3.95	3.95	3.98	4.03	4.10	4.18	4.27	4.36
Solids-not-fat	(%)	9.30	9.20	9.03	8.93	8.87	8.83	8.83	8.83	8.84	8.85	8.85	8.87	8.88	8.91	8.93	8.96
<b>Day's milk:</b>																	
Yield	(lb.)	45.3	45.9	46.9	46.9	46.1	44.1	42.5	41.3	39.9	38.7	37.3	36.1	34.7	33.5	32.1	30.9
Fat	(%)	4.47	4.42	4.24	4.10	3.97	3.84	3.74	3.65	3.63	3.63	3.68	3.74	3.80	3.91	3.98	4.11
Solids-not-fat	(%)	9.36	9.26	9.10	9.00	8.93	8.89	8.89	8.89	8.90	8.91	8.92	8.93	8.94	8.96	9.00	9.03
<b>Wt. of fat (lb.):</b>																	
Morning milk		1.03	1.03	1.00	0.96	0.91	0.84	0.79	0.75	0.72	0.70	0.69	0.68	0.67	0.67	0.66	0.66
Evening "		1.00	1.00	0.99	0.96	0.92	0.85	0.80	0.76	0.73	0.70	0.68	0.67	0.65	0.64	0.62	0.61
Day's "		2.03	2.03	1.99	1.92	1.83	1.69	1.59	1.51	1.45	1.40	1.37	1.35	1.32	1.31	1.28	1.27
<b>Wt. of solids-not-fat (lb.):</b>																	
Morning milk		2.28	2.28	2.29	2.26	2.18	2.12	2.04	1.98	1.93	1.88	1.81	1.76	1.70	1.65	1.59	1.54
Evening "		1.97	1.96	1.96	1.91	1.81	1.61	1.74	1.69	1.63	1.58	1.52	1.46	1.40	1.35	1.30	1.25
Day's "		4.25	4.25	4.27	4.22	4.09	3.93	3.78	3.67	3.56	3.46	3.33	3.22	3.10	3.00	2.89	2.79
Wt. of milk containing 1 lb. of fat (lb.)		22.3	22.6	23.5	24.4	25.2	26.1	26.7	27.4	27.5	27.6	27.2	26.8	26.3	25.5	25.1	24.3
Wt. of total solids (lb.)		6.28	6.28	6.26	6.14	5.92	5.62	5.37	5.18	5.01	4.86	4.70	4.57	4.42	4.31	4.17	4.06
Percentage of fat in total solids		32.4	32.4	32.3	31.3	30.9	30.1	29.1	29.1	29.0	28.8	29.2	29.5	29.9	30.4	30.7	31.3

## PRELIMINARY ANNOUNCEMENT OF THE FIRST INTERNATIONAL CONGRESS OF SOIL SCIENCE.

IN accordance with the decision of the Fourth International Conference of Soil Science that met in Rome, in May 1924, the First Congress of the International Association of Soil Science, then organized, will convene on June 13, 1927, in Washington, D.C. The Congress will be followed by a field excursion to visit the various important soil belts in the country. Opportunity will also be given to the delegates to acquaint themselves with various Agricultural Industries, some of the leading Agricultural Experiment Stations, and in general with the agricultural resources of the United States.

The Association is made up of the following six International Commissions:

### *I. Commission on Soil Physics.*

*Chairman:* Dr V. NOVAK, Chef de l'Institut Pédologique, Kvetná 19, Brno, Czechoslovakia.

### *II. Commission on Soil Chemistry.*

*Chairman:* Prof. Dr A. DE' SIGMOND, Technische Hochschule, Szent-Gellért tér 4, Budapest, Hungary.

### *III. Commission on Soil Bacteriology.*

*Chairman:* Prof. Dr JULIUS STOCKLASA, Professor an der Böhmischen Technischen Hochschule und Direktor der Staatlichen Versuchsstation, Vinohrady, Prague, Czechoslovakia.

### *IV. Commission on Soil Fertility.*

*Chairman:* Prof. Dr E. A. Mitscherlich, Pflanzenbau-Institut der Universität, Tragheimerkirchenstrasse 83, Königsberg, Germany.

### *V. Commission on Nomenclature, Classification and Cartography.*

*Chairman:* Prof. C. F. MARBUT, Bureau of Soils, Department of Agriculture, Washington, D.C.

### *Sub-Commission on the Preparation of the Cartography of Europe.*

*Prof. Dr H. STREMMER*, Mineralogisch-Geologisch Institut der Technischen Hochschule, Neptunstrasse 14, Danzig, Germany.

### *VI. Commission on the Application of Soil Science to Land Cultivation.*

*Chairman:* Dr J. GIRSBERGER, Kultur-Ingenieur des Kantons Zurich, Kaspar Escherhaus, Zurich, Switzerland.

The American representatives of these Commissions are:

- I. Dr C. DAVIS, Bureau of Soils, Washington, D.C.
- II. Dr M. M. MCCOOL, East Lansing, Michigan.
- III. Dr S. A. WAKSMAN, New Brunswick, N.J.
- IV. Prof. D. R. HOAGLAND, Berkeley, California.
- V. Dr C. F. MARBUT, Bureau of Soils, Washington, D.C.
- VI. Dr S. H. MCCRORY, Bureau of Agricultural Engineering, Washington, D.C.

Each Commission is now working on the preparation of its own Programme. Some of the sessions will be devoted to the Congress as a whole or to combined meetings of more than one Commission, while a number of sessions (5 to 8) will be devoted to the special sessions of each Commission.

The Programme of each Commission will consist of papers presented by invitation by outstanding investigators in the respective fields, and of papers presented by various workers in the different branches of Soil Science, by members or non-members of the Association. Titles of the papers to be presented and brief abstracts in English, French and German should be sent on or before December 1st, either to the respective Chairman, or to the American Representative of the Commission, where the paper is to be presented, or to the President of the Association, who will have the paper forwarded to the Chairman of the corresponding Commission.

This Congress will bring together in America, for the first time in its history, all those who are interested in the different problems of soil classification, soil analysis, fertilization and treatment, as well as the relation of the soil to plant growth. Extensive exhibits of various soil types (monolithic columns, in respective horizons) from Europe and America, apparatus used in soil analyses, of the soil microflora and microfauna, etc., will be held during the Congress.

DR J. G. LIPMAN,  
*President, International Society of Soil Science*  
*New Brunswick, N.J.*

DR D. J. HISSINK,  
*Secretary, Groningen, Holland.*





## THE CHEMISTRY OF BASIC SLAGS.

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## INTRODUCTION.

THE two outstanding problems in connection with basic slags are, first, the nature of the lime compounds other than phosphates and their effects on the soil and plant; and second, the nature of the phosphate. The first of these has been discussed in a recent paper by Williams<sup>(1)</sup>. References are there made to the work of Barnette<sup>(2)</sup>, Voelcker<sup>(3)</sup> and McArthur<sup>(4)</sup>. The general conclusion is that basic slags have a distinct fertilising value by reason of their lime content. McArthur in particular, attributes this to the presence of lime as dicalcium silicate.

Williams's experiments showed that the addition of a low-grade slag can increase considerably the amount of exchangeable calcium in soils and also the amount of calcium soluble in water containing carbonic acid.

The second of these problems, namely the chemical nature of the phosphates present, has been studied by Stead<sup>(5)</sup>, who concluded that the most soluble phosphate was a silico-phosphate represented by  $5\text{CaO} \cdot \text{P}_2\text{O}_5 \cdot \text{SiO}_2$ . Morrison<sup>(6)</sup> also from his experiments concluded that the ratio of the lime to the phosphoric acid was 5 : 1.

In the low-soluble slags, it is the general opinion that a basic phosphate or tetracalcium phosphate is present.

In a former paper<sup>(7)</sup> reference was made to the method, used by André and Copaux<sup>(8)</sup>, of decarbonating mineral phosphates by boiling them with a solution of ammonium chloride. These authors found in this way that very little of the phosphate becomes soluble.

It was thought that this method would be likely to give important results with basic slags, since the lime compounds other than phosphates might be removed leaving the slags enriched in phosphorus. The method is in some respects similar to the well-known Hissink method for exchangeable bases in soils.

## EXPERIMENTAL.

Preliminary experiments showed that on boiling a few grams of basic slag with a solution of ammonium chloride, much of the slag became soluble and that the amount dissolved increased with the concentration

of the ammonium chloride solution; also there was a continuous evolution of ammonia gas.

After many experiments, it was decided to treat weighed quantities of the slags with a boiling solution of ammonium chloride of 10 per cent. strength, for definite periods of time.

#### EFFECT OF AMMONIUM CHLORIDE ON BASIC SLAGS.

The procedure finally adopted was as follows: 10 grams of the slag, which had passed the 100-mesh sieve, were boiled with 1000 c.c. of a 10 per cent. ammonium chloride solution in a large flask fitted with a reflux condenser.

At the end of a definite period of time, such as 24 hours, 48 hours, etc., the contents of the flask were filtered through a Buchner funnel, the slag residues were washed with boiling water, dried, ignited and weighed. The amounts of lime, phosphoric acid and silica in these residues were determined, also the amounts of the same constituents that became soluble, when two grams of the residue were shaken with 500 c.c. of a 2 per cent. solution of citric acid for 30 minutes.

#### *Material soluble in ammonium chloride.*

The filtrate and washings from the slag residues were made to a known volume. Aliquot portions were taken for determinations of lime and phosphoric acid. The calcium was precipitated as oxalate in the presence of acetic acid and then estimated volumetrically.

#### *Low-grade slag.*

	24 hours	48 hours	72 hours	96 hours	Second treat- ment	Original slag
Weight of residual slag	0.488	5.365	5.601	4.892	—	—
Soluble matter %...	35.12	46.77	44.0	51.08	—	—
CaO in solution ...	2.600	3.108	3.047	3.23	—	—
% of total CaO ...	56.95	68.08	66.75	70.7	—	—
P <sub>2</sub> O <sub>5</sub> in solution ...	0.001	0.005	0.004	0.005	—	—
% of total P <sub>2</sub> O <sub>5</sub> ...	0.17	0.57	0.57	0.56	—	—
Residues						
% P <sub>2</sub> O <sub>5</sub> ...	12.33	14.80	14.35	16.62	18.09	8.43
Equivalent to Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> %	26.88	32.26	31.06	36.28	39.43	18.39
CaO % ...	25.64	23.23	22.41	22.26	23.34	45.65
SiO <sub>2</sub> % ...	29.62	32.18	27.4	—	35.65	21.3
Ratio P <sub>2</sub> O <sub>5</sub> /CaO ...	0.48	0.63	0.64	0.74	0.77	0.18
Citric soluble, 2 gm.						
P <sub>2</sub> O <sub>5</sub> ...	0.121	0.1617	0.1543	0.1726	0.1518	0.0618
CaO ...	0.2904	0.2496	0.2323	0.2365	0.2310	0.6016
SiO <sub>2</sub> ...	0.0725	0.0075	0.0075	0.002	0.042	0.22
Ratio soluble/total P <sub>2</sub> O <sub>5</sub> ...	49.8	54.0	54.0	51.0	43.6	36.6
Ratio P <sub>2</sub> O <sub>5</sub> /CaO ...	0.41	0.64	0.66	0.73	0.65	0.10

*High-grade slag.*

	24 hours	48 hours	72 hours	96 hours	Second treat- ment	Original slag
Weight of residual slag ...	6.536	5.96	4.149	4.797	3.445	—
Soluble matter %...	34.64	40.40	58.51	52.03	65.55	—
CaO in solution ...	1.750	2.177	3.336	3.003	—	—
% of total CaO ...	38.2	47.6	72.9	65.6	—	—
P <sub>2</sub> O <sub>5</sub> in solution ...	0.024	0.1416	0.547	0.396	—	—
% of total P <sub>2</sub> O <sub>5</sub> ...	1.7	10.0	38.9	28.2	—	—
Residues						
% P <sub>2</sub> O <sub>5</sub> ...	21.00	20.88	19.81	20.45	17.59	14.04
Equivalent to Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> %	45.78	45.52	43.18	44.58	38.36	30.60
CaO % ...	42.15	40.40	29.76	33.06	20.80	45.74
SiO <sub>2</sub> % ...	10.92	10.23	10.90	11.79	15.10	9.64
Ratio P <sub>2</sub> O <sub>5</sub> /CaO ...	0.5	0.5	0.66	0.62	0.84	0.30
Citric soluble, 2 gm.						
P <sub>2</sub> O <sub>5</sub> ...	0.347	0.344	0.308	0.333	0.218	0.245
CaO ...	0.654	0.694	0.448	0.527	0.248	0.802
SiO <sub>2</sub> ...	0.097	0.135	0.055	0.062	—	0.095
% soluble P <sub>2</sub> O <sub>5</sub> ...	82.6	82.5	77.7	81.4	61.6	87.4
Ratio P <sub>2</sub> O <sub>5</sub> /CaO ...	0.53	0.50	0.68	0.63	0.87	0.30

The phosphoric acid was estimated after evaporation and ignition (to volatilise the large amount of ammonium chloride), according to the procedure recommended by Prescott.

The experiments were carried out chiefly on two slags. One was a low-soluble slag containing 18 per cent. phosphate reckoned as tricalcium phosphate and the other was a high-soluble slag containing 30 per cent. phosphate. The results are given in the tables above.

## DISCUSSION OF RESULTS.

Each table is divided into three sections. In the first section there is given the weight of slag left, out of the 10 gm. taken, and the amounts of lime and phosphoric acid found in the solution.

In the second section are given the percentages of lime, phosphoric acid and silica found in the residues, also the ratio of phosphoric acid to lime.

In the third section are given the actual amounts found in the citric acid solution.

It will be observed that more material is removed from the low-grade slag in 48 hours than in 72 hours, and more in 72 hours than in 96 hours from the high-soluble slag. This is probably due to the difficulty of keeping all the particles in intimate contact with the solution during the long period of boiling; the slag tends to remain at the bottom of the flask. It may also be due to the lack of uniformity in the slag, and to the fact that it was not always possible to maintain continuous boiling for so long a period.

*Soluble Matter.*

In each case more than a third of the slag goes into solution in the first 24 hours; the low-grade slag is more rapidly attacked at first but with longer periods of contact with the ammonium chloride solution the amount of substance removed from the high-soluble slag is greater, reaching 58 per cent. in 72 hours.

This is owing to the larger percentage of calcium silicate in the low-grade slag. In this slag very little phosphoric acid goes into solution, but a large amount of the total lime present is rapidly removed. The phosphates in the high-soluble slag are attacked to a much greater extent since there is less calcium silicate present. The amount of lime that becomes soluble in 72 hours is as much as 72.9 per cent. of the total lime content. The figures show that calcium compounds other than phosphates are most readily attacked, especially calcium silicate.

*The residues from the high-soluble slag.*

The percentages of phosphoric acid in the residues from the high-soluble slag after 24, 48, 72 and 96 hours are almost the same. The amounts of silica, too, are almost constant. There is a marked change, however, in the percentage of lime in the residues after 48 hours' treatment.

The molecular proportions of lime, phosphoric acid and silica in these residues are as follows:

24 hours' residue	CaO : P <sub>2</sub> O <sub>5</sub> : SiO <sub>2</sub>	5.1 : 1.0 : 1.23
48        "	"	4.9 : 1.0 : 1.15
72        "	"	3.8 : 1.0 : 1.29
96        "	"	4.09 : 1.0 : 1.3

This would indicate that the phosphate present in the residues after 24 and 48 hours' treatment has the formula 5CaO . P<sub>2</sub>O<sub>5</sub> . SiO<sub>2</sub> and in the other residues the formula 4CaO . P<sub>2</sub>O<sub>5</sub> . SiO<sub>2</sub>. The proportion of silica is somewhat higher than is required by these formulae. This would be expected since some of the silica in the residue results from the decomposition of the calcium silicate present in the original slag.

The constancy of the silica in the residues is somewhat surprising and must be due to the solution of the silico-phosphate in the ammonium chloride as a whole. If none of the silica went into solution, then the percentage of silica in the residues should show a gradual increase and the residue after 24 hours should contain as much as 13.2 per cent., instead of the 10.9 actually found.

It is only after a second treatment that the silica content shows a

marked increase. The residue after 72 hours' treatment was submitted to a further treatment with fresh ammonium chloride solution. A further loss of lime occurred. The percentages of lime, phosphoric acid and silica in the material left are given in the column marked "second treatment." The molecular proportions of these constituents are 3.0 : 1.0 : 2.12.

For reasons that will be given later the silica in this residue is thought to be uncombined and the phosphate present is tricalcium phosphate.

*The solubility of the slag residues in 2 per cent. citric acid.*

From the figures given in the table under the heading "citric soluble," the molecular proportions of lime, phosphoric acid and silica dissolved by the citric acid have been calculated. They are:

24 hours' residue	CaO : P <sub>2</sub> O <sub>5</sub> : SiO <sub>2</sub>	4.8 : 1.0 : 0.66
48        "	"	5.12 : 1.0 : 0.93
72        "	"	3.7 : 1.0 : 0.42
96        "	"	4.02 : 1.0 : 0.44
Second treatment	"	2.9 : 1.0 : nil.

It will be observed in the table that the percentage of soluble phosphoric acid is the same for the 24 and 48 hours' residues, namely 82.5, and is lowest for the residue after a second treatment, being 61.6. Taking this fall in solubility into consideration and also the low values of the soluble silica in the 72 and 96 hours' residues, and the absence of soluble silica in the citric acid solution from the residue after a second treatment, it is concluded that the silica in this residue is uncombined and the phosphate present tricalcium phosphate, also that the residues after 72 and 96 hours' treatment contain mixtures of the silico-phosphate 5CaO . P<sub>2</sub>O<sub>5</sub> . SiO<sub>2</sub> and tricalcium phosphate 3CaO . P<sub>2</sub>O<sub>5</sub>.

*The residues from the low-grade slag.*

It will be seen that there is a gradual rise in the amount of phosphoric acid in the residues as the period of contact increases. The lime content on the other hand soon becomes almost constant. Each residue contains a very high percentage of silica.

The molecular proportions of lime, phosphoric acid and silica are as follows:

24 hours' residue	CaO : P <sub>2</sub> O <sub>5</sub> : SiO <sub>2</sub>	5.3 : 1.0 : 4.9
48        "	"	4.0 : 1.0 : 5.3
72        "	"	4.0 : 1.0 : 4.5
96        "	"	3.3 : 1.0 : 4.5
Second treatment	"	3.2 : 1.0 : 4.5

It is seen from these figures that the residues after 48 and 72 hours contain a tetracalcium phosphate. The large amount of silica present is uncombined as will be evident when the solubility in citric acid is considered. The residue after 24 hours contains the greatest amount of lime; some of this is still combined with silica, since after a further treatment the lime content becomes practically constant. The analysis of the residue after 96 hours shows that the tetracalcium phosphate is slowly decomposed forming the tricalcium phosphate. Even after a second treatment with fresh ammonium chloride solution, there is much tetracalcium phosphate present. This can be seen from the values of the ratio  $P_2O_5 : CaO$ ; the theoretical value of this ratio for tetracalcium phosphate is 0.63, and for tricalcium phosphate 0.84, the values actually obtained are 0.74 for the 96 hours' residue and 0.77 for the residue after a second treatment.

*The solubility of the low-grade slag residues in citric acid.*

The phosphate in the original slag has a solubility of 36.6, the removal of the lime by 24 hours' treatment causes the solubility of the phosphate to rise to nearly 50 per cent. A further rise to 54 per cent. takes place after the next 24 hours' treatment; with more prolonged treatment a slight fall in solubility occurs.

It will be observed that but little silica is dissolved by the citric acid, hence it is concluded that the tetracalcium phosphate in this slag is not combined with silica.

The various residues from this slag were yellowish red in colour after ignition. This is caused by the combination of the iron oxide with some of the silica. The residues from the high-soluble slag were almost white, except that obtained after a second treatment, which was reddish brown owing to the large amount of oxides of iron and manganese present.

*The action of hydrofluoric acid on the slag residues.*

About 0.5 gm. of each residue was treated with hydrofluoric acid in a platinum crucible, the acid was evaporated and the residue ignited; the residues from the low-grade slag always showed a loss in weight, but those from the high-soluble slag always showed an increase. This is seen from the table below:

Low-grade slag	Loss %	High-soluble slag	Gain %
Original slag	8.35 (gain)	Original slag	20.0
24 hours' residue	17.2 loss	24 hours' residue	8.68
48     "	16.6     "	48     "	12.54
72     "	14.7     "	72     "	19.05
96     "	25.5     "	96     "	10.33
Second treatment	20.0     "	Second treatment	5.3

At first sight it would appear as if the two slags behaved quite differently, but if the different amounts of silica in the two slags are considered, it will be seen that the action is the same in the two cases. In the low-grade slag residues, the loss of silica by the action of the hydrofluoric acid is only partly counteracted by the fixation of fluorine by the phosphate and bases present, so that there is a net loss in weight; on the other hand with the residues from the high-soluble slag, the loss of silica is more than counteracted by the fixation of fluorine by the larger amount of phosphate so that there is a net gain.

#### GENERAL CONCLUSIONS.

The experiments described show that basic slags contain calcium silicates which are readily decomposed and thus support the conclusions of Williams, that they can supply large amounts of exchangeable calcium. It is suggested that in the soil, after a period of time which will depend on conditions such as organic matter, rainfall and climate, the silicate gives up its lime leaving the phosphate more open to attack. In the case of high-soluble slags the period of time may be very short since they have small amounts of calcium silicate but a large amount of easily soluble phosphate. It is possible that such a slag on very acid soils, applied in relatively small quantity might not supply sufficient exchangeable calcium, whereas a low-soluble slag in greater quantity might do so.

The conclusion of Stead that the high-soluble phosphate is a silico-phosphate is confirmed, and also Morrison's figure for the ratio of lime to phosphoric acid.

The 30 per cent. slag was brought to a phosphate content of 45.78 per cent. by 24 hours' treatment, the other residues contained less phosphate. The 18 per cent. slag was more gradually enriched in phosphorus and after 72 hours' treatment and a further boiling with fresh ammonium chloride, it was brought to 39.42 per cent. phosphate. It might be possible by using a hot brine to convert slags of low phosphate content into material of high phosphate content. Such residual slags, however, would not have a high solubility and would contain large amounts of iron and manganese oxides and silica. The fall in the solubility of the phosphate in the residue from the high-soluble slag after a second treatment is believed to be due to the effect of the iron oxide present, which amounts to more than 20 per cent.



## FLUORSPAR SLAGS.

The experiments on basic slags and mineral phosphates described in former papers, have now been extended to fluorspar slags. The slags used were very kindly supplied by Mr F. Bainbridge, of the Skinningrove Iron Works and were those used by him in his investigations several years ago.

The samples were taken from a cast specially made. Sample No. 1 was made without addition of fluorspar. Sample No. 2 was taken after large additions of spar had been made. Sample No. 3 was made in another special cast, to which very large additions of spar were made to ensure the conversion of the phosphate to apatite.

As in the former work, the ratio of phosphate soluble in 2 per cent. citric acid to total phosphate present has been determined. The results are given below. For purposes of comparison the solubilities of apatite and of calcium pyrophosphate over the same range of total phosphate have been determined.

*Non-fluorspar slags. Samples A and B. Total  $P_2O_5$ . A, 13.67 %. B, 13.55 %.*

Sample A			
Citric soluble $P_2O_5$ weight taken	$P_2O_5$ present	$P_2O_5$ soluble	% soluble
1 gm.	0.1367 gm.	0.0667 gm.	48.8 gm.
3	0.4101	0.0897	21.8
5	0.6835	0.1362	19.9
8	1.0936	0.1905	17.4

Sample B			
1 gm.	0.1355 gm.	0.0592 gm.	43.7 gm.
3	0.4065	0.0937	23.0
5	0.6775	0.1335	19.7
8	1.084	0.1884	17.4

*Fluorspar slags. Total  $P_2O_5$ . Sample 1, 14.66 %. Sample 2, 14.75 %.*

Sample 1			
Citric soluble $P_2O_5$ weight taken	$P_2O_5$ present	$P_2O_5$ soluble	% soluble
1 gm.	0.1466 gm.	0.0255 gm.	17.4 gm.
3	0.4398	0.0291	6.61
5	0.733	0.0372	5.07
8	1.1728	0.0504	4.30

Sample 2			
1 gm.	0.1475 gm.	0.0219 gm.	14.9 gm.
3	0.4425	0.0299	6.5
5	0.7375	0.0381	5.17
8	1.180	0.0495	4.20

*Fluorspar slag when excess of fluorspar added. Total  $P_2O_5$ . 11.58 %.*

Citric soluble $P_2O_5$ weight taken	$P_2O_5$ present	$P_2O_5$ soluble	% soluble
1 gm.	0.1158 gm.	0.0167 gm.	14.4 gm.
3	0.3474	0.0185	5.3
5	0.5790	0.0213	3.7
8	0.9264	0.0246	2.6

*Apatite. Total  $P_2O_5$ . 18.93 %.*

Citric soluble $P_2O_5$ weight taken	$P_2O_5$ present	$P_2O_5$ soluble	% soluble
1 gm.	0.1893 gm.	0.0118 gm.	6.27 gm.
3	0.2839	0.0161	5.69
5	0.3786	0.0197	5.20
8	0.7572	0.0256	3.40

*Calcium pyrophosphate. Total  $P_2O_5$ . 55.44 %.*

Citric soluble $P_2O_5$ weight taken	$P_2O_5$ present	$P_2O_5$ soluble	% soluble
0.5 gm.	0.277 gm.	0.075 gm.	27.0 gm.
0.75	0.415	0.1092	26.5
1.0	0.5544	0.1525	27.0
1.5	0.830	0.213	25.1
3.0	1.663	0.2946	17.7
5.0	2.772	0.429	15.5
8.0	4.435	0.630	14.2

The phosphate in the non-fluorspar slag has the greatest solubility although the figures show that it is only 20 per cent. soluble when 5 gm. are taken for the test. If the figures for citric soluble phosphate are plotted against total phosphate, then the curve for the first fluorspar slag lies very near that for apatite, while the curve for the second fluorspar slag, in which an excess of fluorspar had been employed, is actually identical with the apatite curve. This confirms the conclusions of Bainbridge<sup>(9)</sup> that the phosphate present in these slags has the same composition as apatite.

#### SUMMARY.

1. Two basic slags differing widely in solubility and phosphate content have been treated for long periods with boiling ammonium chloride solution.

2. The low-grade slag lost 70 per cent. of the total lime and very little phosphoric acid; a residue with nearly 40 per cent. phosphate was obtained from an 18 per cent. slag.

3. The high-soluble slag also lost a large percentage of the total lime but large amounts of phosphoric acid were also found in solution. The residue from the 30 per cent. slag after 24 hours' treatment contained nearly 46 per cent. phosphate.

4. It is shown that a silico-phosphate is present in the high-soluble slag, but not in the low-grade slag.

5. The solubility in citric acid of the phosphates in the various residues has been determined.

6. The value of basic slags in supplying exchangeable calcium is discussed.

7. The effect of hydrofluoric acid on the slag residues has been investigated.

8. Experiments with fluorspar slags are described which confirm the view that the phosphate present is fluorapatite of very low solubility in citric acid.

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# THE DETERMINATION OF NITRATES IN PLANT MATERIALS.

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DURING a storage experiment with mangels carried out in 1924-25 by the writer, an attempt was made to estimate the nitrate content of the roots at different times. Many methods have been proposed, from time to time, for the determination of nitrates, but very few have been found free from objection, particularly when used in the presence of organic matter. Sutton writes (*Volumetric Analysis*, 10th ed. 1911, p. 271): "The accurate determination of nitric acid in combination presents great difficulties and can only be made by indirect means.... Very few of them can be said to be simple, but it is feared that no simple process can ever be obtained for the determination of nitric acid in many of its combinations." In looking around for a process which might be used with mangel juice, the Valmari-Devarda method, based on distillation with magnesia and Devarda's alloy, and recently recommended by Gallagher(1), attracted attention, and as it seemed to offer considerable advantages both of ease and rapidity, was tried for these estimations. The results were, however, disappointing; it was found impossible to obtain concordant duplicates, and the blanks made by distillation with magnesia alone, also varied greatly, and were moreover often nearly as great as the amounts of nitrate to be estimated. When very small amounts of nitrate had to be determined, as was usually the case late in the storage period, the high relative value of the blanks was in itself a sufficiently serious objection to the method, apart altogether from its lack of consistency.

At the time these estimations were made, it seemed to the writer that the very indefinite partial decompositions of proteins, certain amino acids, etc., brought about with liberation of ammonia, by the action of magnesia at the boiling-point of water might, perhaps, account for the discrepancies in both blanks and estimations. If that were so, it seemed that the method might be rendered serviceable by a suitable choice of alkali and temperature so as to avoid these decompositions. It was decided therefore to investigate the reducing action of Devarda's alloy under conditions of alkalinity and temperature comparable to those

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which had been proved satisfactory for the estimation of ammonia in plant products. A reduction method devised along these lines would have the advantage that the blanks would represent the ammonia actually present in the juice (assuming ammonia-free reagents), instead of the amount of ammonia evolved by partial decomposition of nitrogenous substances under arbitrary conditions. Two methods, which had been shown to be satisfactory for the determination of ammonia in the presence of organic matter, were available, and seemed likely to provide conditions suitable for use with Devarda's alloy. These were (i) distillation *in vacuo* with lime at 45–50° C., used by Van Slyke<sup>(2)</sup>, and (ii) the aeration method. The first of these seemed to offer most advantages for investigation. It appeared not improbable that as the very faintly alkaline reaction of magnesia sufficed to bring about reduction with Devarda's alloy at 100° C., the much greater alkalinity of calcium hydroxide might prove effective at the lower temperature of 45–50° C. Experiments, which will be described later, were made to test this hypothesis, and showed that reduction under these conditions did in fact bring about quantitative recovery of nitrate from pure solutions, but failed to do so in the presence of organic matter. At about this stage of the work it was discovered that the Valmari-Devarda method, using magnesia, had been already investigated by Allen<sup>(3)</sup>, and found to be unsuitable for the determination of nitrates in presence of organic matter. Replacement of the magnesia by sufficient sodium hydroxide to make the solution *N*/10 was shown by him to lead to very satisfactory estimations. Even Allen's method suffers, however, and perhaps more than that in which magnesia is employed, from the drawback that the blanks represent, not only ammonia present in solution as ammonium salts, but also that liberated from various nitrogenous substances by the action of the hot alkali. The disadvantages of this are obvious, particularly, as has been emphasised already, when the amounts of nitrate present are small. It was hoped to obviate this difficulty, as stated earlier, by carrying out the Devarda distillation *in vacuo*, at 45–50° C., in the presence of lime as alkali. But as this object was found to be unattainable in presence of organic matter it was considered advisable to investigate more fully the estimation of nitrates by some other methods. It may be stated at the outset that attention was directed only to reduction methods which involve alkaline solutions. Reduction methods in general have the advantage of leading to the production of a substance, ammonia, which can be estimated with great accuracy, and ease. Moreover it has been pointed out by Gallagher, that reduction in acid solution is

objectionable with plant materials, owing to the danger of evolution of free nitrogen by interaction of amino groups and the nitrous acid presumed to arise as an intermediate product in these reductions. Alkaline reductions seemed therefore best suited to determinations of nitrates in presence of nitrogenous organic matter. The result of the work, which will now be described, has been the development of two satisfactory methods of estimating nitrates in plant juices and similar materials. The first depends on the use of Devarda's alloy in cold weakly alkaline solution, the second on the reducing power of titanous hydroxide (to which substance Knecht first drew attention), under conditions which obviate any risk of decomposing organic matters.

*Distillation in vacuo with Devarda's alloy and calcium hydroxide, etc.* Van Slyke has shown (*loc. cit.*) that ammonia can be determined in the presence of proteins and their products of hydrolysis by distillation *in vacuo* at 45–50° C., with milk of lime. For the reasons given earlier it was thought possible that the addition of finely powdered Devarda's alloy to a nitrate solution followed immediately by this type of distillation would enable reduction (and concurrent removal of the ammonia produced) to take place, without liberating ammonia from other substances than ammonium salts. A trial experiment in which a known solution of nitric acid was distilled for 45 minutes in this fashion showed quantitative reduction to ammonia. The addition of a known amount of mangel juice to the nitrate solution led, however, to an almost complete failure of the reduction, only a minute proportion of the nitrate being recovered as ammonia. Evidently the presence of organic matter in the juice interfered greatly with the reduction. The removal of part of the protein substances by precipitating agents such as alcohol, copper hydroxide, alum and basic lead acetate led to a slight improvement in the recovery of ammonia, but the amounts obtained still fell far short of the theoretical (see Table I).

Table I. *Distillation in vacuo at 45–50° C., with Devarda's alloy and calcium hydroxide.*

Nitrate solution				Ammonia as c.c. N/10	
				Expected	Recovered
1.	10 c.c. N/10 HNO <sub>3</sub>	...	...	10.0	9.95
2.	25 c.c. mangel juice	...	...	13.6 (approx.)	2.3
3.	" (cleared with alcohol)	...	...	13.6	3.5
4.	" (cleared with basic lead acetate)	...	...	13.6	1.3
5.	" (cleared with copper hydroxide)	...	...	13.6	2.3

It seemed possible that the reduction might be improved by using, instead of calcium hydroxide, some alkali which could dissolve the

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aluminium hydroxide formed during reduction and thus constantly expose a fresh surface of the alloy. Sodium hydroxide, in amount sufficient to render the solution *N*/20 (*i.e.* approximately the strength of saturated lime water), was accordingly tried, and was found to give greatly improved nitrate recovery from mangel juice; in one instance, at least, the recovery that was expected. Its use was, however, rendered impracticable by excessive frothing which could not be controlled even by the use of the froth trap described by Davis(4). Barium hydroxide then suggested itself as an alkali unlikely to suffer from this disadvantage of forming soluble soap-like compounds with any of the juice constituents, and capable of use in higher concentrations than calcium hydroxide, and was investigated at several concentrations. The results obtained by its use, and those previously described in which other alkalis were employed, are shown in Table II.

Table II. *Distillation in vacuo of mangel juice (cleared with alcohol) with Devarda's alloy and various alkaline solutions.*

Nitrate solution				Ammonia as c.c. <i>N</i> /10	
				Expected	Recovered
20 c.c. juice A	...	...	<i>N</i> /20 NaOH	—	5.7
20 "	+ 10 c.c. <i>N</i> /10 HNO <sub>3</sub>	...	"	15.7	16.25
24 c.c. juice B	...	...	<i>N</i> /10 Ba(OH) <sub>2</sub>	—	8.3
24 "	+ 5 c.c. <i>N</i> /10 HNO <sub>3</sub>	...	"	13.3	13.2
20 "	+ 10 c.c. <i>N</i> /10 HNO <sub>3</sub>	...	"	16.9	15.85
20 "	+ 10 c.c. <i>N</i> /10 HNO <sub>3</sub>	...	<i>N</i> /5 Ba(OH) <sub>2</sub>	16.9	16.25

The use of barium hydroxide is seen to have led to improved nitrate recovery, increasing with the concentration of the alkali, but quantitative recovery would probably require the use of concentrations so high as to involve liberation of ammonia from protein and similar materials, and would thus defeat the purpose of the method. Investigation of the possibility of direct estimation of nitrate by comparatively rapid vacuum distillation with Devarda's alloy in alkaline solution at moderate temperature was finally abandoned, and attention was directed to the possibilities of carrying out reductions with Devarda's alloy in cold weakly alkaline solutions.

### *Reduction with Devarda's alloy in the cold.*

Many types of cold alkaline reduction have been used from time to time for the estimation of nitrates in the presence of organic matter. Reduction with aluminium strips in 1 per cent. alkali has been recommended by Burgess(5), and although criticised by Allen(3), has more recently been used with fair success by Van Wijk(6), for the estimation

of small amounts of nitrate such as occur in soils. It seemed probable that the finer state of division of Devarda's alloy, coupled with its greater reducing activity would enable it to be used in the cold with greater certainty of quantitative reduction, and perhaps in much lower concentration of alkali, than was possible with aluminium. A number of nitrate estimations were made on mangel juice using 1 gram of the alloy (ground to pass a 100-mesh slag sieve) in different concentrations of sodium hydroxide. With  $N/20$  alkali, reduction was found to be fairly but not quite complete, but with  $N/10$  alkali it appeared to be practically quantitative (see Table III).

Table III.

Nitrate solution					Concentration of sodium hydroxide	Ammonia as c.c. <i>N</i> /10	
						Expected	Recovered
24 c.c. juice <i>C</i>	...	...	...	...	<i>N</i> /20	—	18.5
24 „	+ 10 c.c. <i>N</i> /10 $HNO_3$	...	...	...	„	28.5	26.9
24 „	+ 15 c.c. <i>N</i> /10 $HNO_3$	...	...	...	„	33.5	32.25
25 c.c. juice <i>D</i>	...	...	...	...	<i>N</i> /10	—	15.05
12.5 „	+ 5 c.c. <i>N</i> /10 $HNO_3$	...	...	...	„	12.5	12.35
10 c.c. juice <i>E</i>	...	...	...	...	„	—	4.25
10 „	+ 10 c.c. .89 <i>N</i> /10 $HNO_3$	...	...	...	„	13.15	13.1
10 „	+ 15 „	„	„	„	„	17.6	17.5
20 „	+ 10 „	„	„	„	„	17.4	17.25

The reductions were carried out in a vessel similar to that proposed by Van Wijk (*loc. cit.*), consisting of an Erlenmeyer flask (cap. 700 c.c.), closed with a rubber stopper through which passed a small thistle funnel containing a little glass wool. In the procedure adopted, the solution to be reduced (mangel juice cleared by the addition of an equal volume of alcohol and then filtered) was placed in the flask, and diluted to about 250 c.c. Three c.c. of 30 per cent. sodium hydroxide, *i.e.* sufficient alkali to make the solution approximately  $N/10$  and 1 gram of finely ground Devarda's alloy were then introduced and the stopper inserted. Reduction was allowed to proceed overnight. Moistening the glass wool with a few drops of  $N/10$  sulphuric acid served to prevent any loss of ammonia in the evolved hydrogen. Next morning the acid on the glass wool was washed into the flask, the stopper removed, 5 c.c. of concentrated hydrochloric acid run in, and the flask immediately closed by a well-fitting rubber stopper. It was then shaken until the dense cloud of ammonium chloride, produced on addition of the hydrochloric acid, had dissolved, when its contents were transferred to an R.B. flask and distilled *in vacuo* at 45–50° C., with 2 gm. of ignited lime. The apparatus for distillation followed more or less the lines of that used by Van Slyke



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(*loc. cit.*), with some slight alterations to suit the special conditions. An R.B. long-necked flask of about 1.5-litre capacity was used to contain the reduced solution, and was closed by a 3-holed rubber stopper through which passed a fine capillary tube, tap-funnel, and splash-head. The last was connected to a fairly long Liebig condenser. The receiver consisted of a 400 c.c. distilling flask, the side tube of which passed well down into a second much smaller distilling flask, which was connected through its side tube with the water pump. The greater part of the receiving acid was placed in the larger distilling flask, and a small amount (usually 5 c.c.), diluted with water to about 50 c.c., in the smaller. The small flask therefore served as a bubbler or trap to prevent loss of ammonia, most of which was, of course, retained in the first receiver. About 150 c.c. of distillate were collected in a measured amount of  $N/10$  sulphuric acid and the ammonia determined by titration, using methyl red as indicator. Some of the results obtained by this method, are shown in the lower part of Table III and are seen to be very satisfactory. This opinion has been confirmed as a result of the frequent use of the method during the past year, and also by a comparison of it with another method of nitrate estimation, based on the use of titanous hydroxide, which will now be discussed.

### *Estimation of nitrate in plant juice with titanous hydroxide.*

For the estimation of nitrate in plant products, by reduction, it is desirable to use only methods which involve alkaline solutions. The use of acid reducing agents has been found to give low results by Gallagher (1), who attributes the loss of nitrogen to interaction of amino and similar groups present in proteins, etc., with the nitrous acid formed as an intermediate stage in the reduction. Knecht (7) has shown that titanous hydroxide, which is formed as a voluminous white precipitate by the addition of alkali to the chloride, or sulphate, spontaneously and rapidly decomposes with evolution of hydrogen, and can be used to determine nitrates. As the reduction takes place in alkaline solution, it seemed that it might offer advantages for use with plant juices, after suitable modification. In the procedure recommended by Knecht for the reduction of a pure nitrate, *e.g.* sodium nitrate, 0.1 gram of the nitrate is treated with excess of sodium hydroxide in a copper flask, and 20 c.c. of commercial titanous sulphate or chloride are then run in. The mixture is diluted with water and the ammonia distilled off and titrated. As described above this method is much too drastic for use with plant products, if secondary decompositions are to be avoided, but is capable of being employed if

the alkalinity and the conditions of distillation are modified to correspond with those used in the Devarda reduction. The reducing properties of titanous hydroxide are due to its spontaneous decomposition with evolution of hydrogen, and do not appear to depend on the presence of excess alkali, provided that sufficient is added to precipitate completely all the titanous chloride as hydroxide. The evolution of ammonia which might result from the prolonged action of strong alkali on plant extracts is therefore obviated. Furthermore the reduction has been found by Knecht to be almost instantaneous, at room temperature, so that the solution need never exceed the comparatively low temperature of 45–50° C. used in vacuum distillation with lime. Thus none of those conditions which have been shown to be safe for the determination of ammonia in plant or animal extracts, appear likely, on theoretical grounds, to affect the efficiency of this method of reduction, a conclusion supported by experiment.

The procedure found to give satisfactory estimation of nitrate in mangel juice is as follows. The quantity of alkali necessary to neutralize and precipitate completely the titanous chloride used, is added to the plant juice (which has been previously cleared with alcohol) in a 1500 c.c. long-necked R.B. flask. The proper amount of commercial titanous chloride is immediately run in and the whole shaken. (Knecht's figure of 25 c.c. of 15 per cent. commercial titanous chloride for the nitrate in 0.1 gm.  $\text{NaNO}_3$  was found to be approximately correct also for nitrate in the presence of organic matter.) A few c.c. of fairly concentrated calcium chloride solution are next added to bring the residual alkalinity of the solution within safe limits, and the mixture is allowed to stand for five minutes. It is then diluted to about 500 c.c., with an addition of 100 c.c. alcohol to prevent frothing and the ammonia is distilled off, under reduced pressure, at 45–50° C.

The approximate amount of alkali necessary to secure complete precipitation of the titanous hydroxide is obtained by titrating some of the strongly diluted titanous chloride with standard alkali, using phenolphthalein as indicator. The titration is carried to a decided pink. All the reagents used in the reduction are run into the R.B. flask, previously connected up for distillation, through a tap funnel. When, as sometimes happens, larger amounts of titanous chloride and, consequently, of strong alkali also, have to be used, these reagents are added alternately in the correct proportion, in a number of smaller amounts of 20–30 c.c., in order to prevent the solution becoming too strongly alkaline, even for a short time. In most estimations the quantities of reagents employed

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were: 30 per cent. NaOH, 30 c.c.; commercial titanous chloride solution, 20 c.c.; calcium chloride, 10 gm. dissolved in 30–40 c.c. water. It is of course necessary to ensure that the reagents are ammonia-free, or to perform blanks. Considerable amounts of ammonia were found in one of the samples of titanous chloride.

The results of some estimations carried out on mangel juice which had been previously cleared with alcohol are given in Table IV. For comparison, a few estimations made on the same juice by the Devarda method described earlier are appended. The two are seen to be in good agreement.

Table IV. *Reductions with titanous hydroxide.*

						Ammonia as c.c. N/10	
						Expected	Recovered
Nitrate solution							
50 c.c. juice <i>F</i>	...	...	...	...	...	—	17·3
25 "	...	...	...	...	...	8·65	8·65
25 "	+ 5 c.c. N/10 HNO <sub>3</sub>	...	...	...	...	13·65	13·75
25 c.c. juice <i>G</i> (using 20 c.c. comm. TiCl <sub>3</sub> solution)	...	...	...	...	...	—	8·55
25 "	" 40	...	...	...	...	—	8·5
50 "	" 40	...	...	...	...	17·05	16·95
25 "	+ 10 c.c. N/10 HNO <sub>3</sub>	...	...	...	...	18·5	18·6
50 c.c. juice <i>G</i>	...	...	...	...	...	—	16·95
25 "	...	...	...	...	...	—	8·45

(Cold Devarda reductions for comparison.)

In comparing the two methods it may be said that the Devarda reduction is the easier to carry out, but requires the reduction to proceed overnight. The estimation of nitrate by titanous hydroxide occupies about an hour and is therefore advantageous when quick results are needed, but the distillation requires more attention on account of the liability to frothing, and the reagents used are not so likely to be ammonia-free.

### SUMMARY.

1. The Valmari-Devarda method of estimating nitrates (*i.e.* distillation with magnesium oxide and Devarda's alloy) is unsatisfactory in presence of organic matter. Attempts to make it quantitative under these conditions by modifications of alkali and temperature proved unsuccessful.

2. Two new methods for the estimation of nitrate in plant juices are described. The first depends on the use of Devarda's alloy in cold weakly alkaline solutions, the second on the reducing power of titanous hydroxide, under conditions suitable for use with plant products.

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# LABORATORY AND FIELD EXPERIMENTS ON THE USE OF 3 : 5-DINITRO-*o*-CRESOL AND THE SODIUM SALT FOR WINTER SPRAYING.

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(With One Text-figure.)

AN account has been given in a previous paper<sup>(1)</sup> of experiments on the toxicity of 3 : 5 dinitro-*o*-cresol and the sodium salt to the eggs of the moth, *Selenia tetralunaria* Hufn., under laboratory conditions, and to the eggs of the aphid, *Phorodon humuli* Schr., under field conditions. A high degree of toxicity to both insects was shown. The main objects of the work reported in the present paper were (a) to investigate the effect of dinitro-cresol and the sodium salt on the eggs of a number of different insects; (b) to compare the toxicity of dinitro-cresol with that of the sodium salt under different conditions; (c) to ascertain whether the stage of development of the eggs at the time of treatment affected the resistance to the action of dinitro-cresol; (d) to investigate the effect on toxicity of washing treated eggs with water at different times after treatment; and (e) to extend experiments under field conditions to fruit trees and pests other than Hop-Damson Aphid on plums. The work is not complete but sufficient progress has been made to justify discussion of the results obtained.

## LABORATORY EXPERIMENTS.

The insects used in the laboratory experiments were not all species of economic importance and were not necessarily species which could be controlled by the use of ovicidal spray fluids. They were chosen primarily on account of their availability and the relative ease with which they could be reared in captivity so as to give large numbers of eggs under conditions convenient for experimental work. In many cases, it was possible to get the eggs laid on muslin which could then be cut into pieces, each bearing a suitable number of eggs for one test. Care was taken to protect from the activity of parasites and the results are not complicated by the presence of this factor.

The general procedure adopted for carrying out the tests has been described in previous publications(1, 2). Briefly, the eggs were sprayed under controlled conditions in the spraying apparatus(3), allowed to dry, and each lot put up in a separate tube with a muslin cover and kept in the outdoor insectary until hatching took place. Controls were kept under identical conditions. The number of larvae hatched and of unhatched eggs were counted. During the hatching period, whenever possible, the larvae were counted and removed from the tubes each day. Any departures from this procedure are indicated in the appropriate sections.

Dinitro-cresol is only slightly soluble in water and it was therefore necessary to use an organic solvent in making up the fluids for spraying. In most cases, benzene was used in the laboratory experiments, 0.5 gm. dinitro-cresol being dissolved in 2.5 c.c. benzene and the benzene solution emulsified with 1 per cent. saponin solution and made up to 100 c.c. This emulsion was then diluted with 1 per cent. saponin solution to give the concentrations required for the tests. Unless otherwise stated, this method was adopted in the experiments to be described. A commercial "emulsified disinfectant" was also used in some experiments as a solvent for dinitro-cresol. In this case a 10 per cent. stock solution was prepared and the appropriate dilutions made with water; no saponin was added. Repeated control experiments with the solvents showed that the benzene-saponin solution mixtures had no toxicity to eggs of the species used. The "emulsified disinfectant," at the higher concentrations, had some effect on eggs of the less resistant species (see Table II).

Sodium dinitro-cresylate is soluble in water to the extent of about 5 per cent. and the solutions for spraying were prepared by dilution of a solution containing 1 per cent. of the salt and 1 per cent. of saponin with 1 per cent. saponin solution.

#### *Eggs of the Winter Moth (Cheimatobia brumata L.).*

1. A preliminary experiment was made in the winter 1924-25 with eggs of this species, some being on apple twigs and some on the muslin cover of the cage in which the moths were confined. About half the total number of eggs (223) was thoroughly wetted with dinitro-*o*-cresol at a concentration of 0.25 per cent. on January 30, 1925. The remainder were wetted with 1 per cent. saponin solution as control; both lots were kept in the insectary under identical conditions. Hatching of the control eggs began on April 15 and continued till May 9, the young larvae being removed and counted each day; 284 out of a total of 290 eggs, *i.e.*

98 per cent., finally hatched. 79 larvae in all hatched from the treated eggs and of these 67 were found dead within a few hours of emergence; 12 only (*i.e.* 5.4 per cent.) were apparently normal. All the larvae which hatched from the treated eggs came from eggs laid in crevices of the bark on the twigs; none hatched from those laid on muslin. At the concentration used, dinitro-cresol is evidently fatal to the eggs of the Winter moth; when contact between the fluid and the eggs is satisfactory, as with the eggs on the muslin and the majority on the twigs, they are killed outright; when contact is less satisfactory as with some of the eggs on the twigs, development and hatching may not be prevented but most of the larvae, none the less, die immediately after emergence, possibly due to ingestion of a trace of the material during eclosion.

2. Adults were reared from larvae hatching from control eggs in the previous experiment. They did not however pair well in captivity and only about 600 eggs were obtained. These were divided into lots of 50 and sprayed under the usual conditions in the spraying apparatus on March 8, 1926. Details of the treatments and the results are given in Table I. The larvae in the control experiments hatched between March 25 and April 10.

Table I. *Toxicity of 3 : 5-dinitro-o-cresol to eggs of the Winter Moth.*

Treatment	Concentration in gm. per 100 c.c.	No. of eggs treated	No. of eggs not hatched	% of eggs not hatched	% of viable eggs killed (allowing for control)
Controls	—	200	17	8.5	—
Dinitro-cresol (benzene as solvent)	0.25	50	50	100	100.0
	0.2	50	50	100	100.0
„	0.1	50	50	100	100.0
	0.05	50	40	80	78.1
Dinitro-cresol (“emulsified disinfectant” as solvent)	0.25	50	50	100	100.0
	0.2	50	50	100	100.0
	0.1	50	38	76	73.8
„	0.05	50	13	26	19.1

The high toxicity of dinitro-cresol to eggs of the Winter moth was confirmed; 70–100 per cent. were killed at a concentration as low as 0.1 per cent. The toxicity at 0.05 per cent. was apparently lower when the solvent was the “emulsified disinfectant” than when the benzene-saponin emulsion was used; this is probably due to some error; in experiments with eggs of other species, somewhat increased toxicity has generally been noted when this solvent was used (*cf.* p. 167). Evidence with regard to the effect of dinitro-cresol on Winter moth eggs under field conditions is given in another section.

*Eggs of the Purple Thorn Moth (Selenia tetralunaria Hufn.).*

An account has already been published (2) of experiments with dinitro-cresol and its sodium salt on large numbers of eggs of this moth. Dinitro-cresol at 0.15 per cent. and higher concentrations killed 100 per cent. of the eggs, and at concentrations between 0.1 and 0.04 per cent., the percentage killed ranged from 98.8 to 91.9 per cent.; the sodium salt was very slightly, but probably significantly, less toxic. These figures have been many times confirmed; and one series of experiments with the ammonium salt of dinitro-cresol gave results in very close agreement with those for the sodium salt. The potassium and barium salts are apparently considerably less effective but only small numbers of eggs were used in testing these compounds and the figures have not the same reliability as in the other cases.

The Purple Thorn moth has proved to be specially suitable for work of this kind. It has two generations in the year, the adults pair and lay well in captivity and the larvae are comparatively easy to rear in large numbers on hawthorn or hazel. Unfertilised eggs are readily picked out by their colour and the hatch from fertilised eggs is high; for many generations it has not fallen below an average figure of 90–95 per cent. There is little difficulty in protecting from parasites; no parasitisation of eggs has been observed.

*Eggs of the Glasshouse Tomato Moth (Hadena oleracea L.).*

Starting with a few batches of eggs kindly supplied by Mr E. R. Speyer, of the Lea-Valley Experimental Station, several generations of this insect were reared and a good supply of eggs obtained. The earliest batches of eggs laid generally give a high percentage hatch; batches laid later are liable to include a large proportion of unfertilised eggs. The adults pair fairly well in captivity and will lay on muslin. Although a pest in tomato houses, the larvae will not thrive on tomato only (4), but several hundred were reared without great difficulty on *Chenopodium album* and *Plantago major*. A few of the over-wintering pupae were attacked by a fungus—a species of *Isaria*.

The results of tests on the eggs of this species with dinitro-cresol and its sodium salt are given in Table II.

The spraying was done on July 8, when the eggs were already considerably developed; hatching began on July 11 and larvae were counted and removed on July 12, 13, 14, 15, 17 and 19; the final count of unhatched eggs was made on July 22 and 23. The eggs are much more



difficult to count than those of *S. tetralunaria*, because they are often laid in batches of several tiers, and unfertilised eggs are not easily distinguished from the others. The results are definitely less accurate than in the case of *S. tetralunaria*.

The mean figure for the percentage of eggs which did not hatch in the control lots was 9.2 per cent., but there was a wide range (0-29 per

Table II. *Toxicity of 3 : 5-dinitro-o-cresol and its sodium salt to eggs of Hadena oleracea.*

Treatment			No. of eggs sprayed	No. of eggs not hatched	% eggs not hatched	% viable eggs killed (allowing for control)
Substance	Solvent					
<i>Controls.</i>						
Unsprayed	6 lots		367	46	12.5	—
Saponin 1%	5 lots		487	45	9.24	—
Benzene 100-10%	4 tests		297	15	5.05	—
and Saponin 1%			1151	106	9.21	—
<i>Mean control figure</i>						
		(c.c. %)				
Dinitro-cresol	0.2	Benzene	2	186	186	100.0
"	0.1	"	1	140	140	100.0
"	0.05	"	0.5	130	130	100.0
"	0.025	"	0.25	129	60	46.5
"	0.01	"	0.1	67	7	10.4
Dinitro-cresol	0.2	"Emulsified disinfectant"	2	166	136	(82.0)
"	0.1	"	1	200	200	100.0
"	0.05	"	0.5	100	100	100.0
"	0.025	"	0.25	134	110	82.0
"	0.01	"	0.1	65	19	29.1
None	—	"Emulsified disinfectant"	2	89	56	63.0
"	—	"	1	65	13	20.0
"	—	"	0.5	26	10	38.4
"	—	"	0.25	106	0	—
"	—	"	0.1	124	7	5.8
<i>Sodium salt of *</i>						
dinitro-cresol	0.2	Water with 1% saponin	140	140	100.0	100.0
"	0.1	"	94	94	100.0	100.0
"	0.05	"	130	130	100.0	100.0
"	0.025	"	115	20	17.4	9.0
"	0.01	"	94	30	31.9	25.0

\* The percentages of the sodium salt are in all cases calculated as dinitro-cresol.

cent.) in the values for the different lots, probably mainly due to the difficulty of picking out unfertilised eggs. Figures below about 30 per cent. in the last column of the Table (percentage viable eggs killed) are therefore of doubtful significance.

The figures include, as killed, eggs from which larvae hatched and died immediately after emergence. These were rather numerous in this series of experiments. It was noted that where the eggs were in masses

of several layers, larvae hatched in many instances from eggs in the lowest layer, when the remainder were killed at the stage of development reached when the spray was applied. It is probable that eggs in the lowest layer of a mass were not always thoroughly wetted by the spray.

Both dinitro-cresol and sodium dinitro-cresylate were completely toxic at concentrations of 0.05 per cent. and above. Lower concentrations had little effect except where the "emulsified disinfectant" was used as a solvent, in which case 0.025 per cent. killed 80 per cent. of viable eggs. This difference in effect cannot be attributed to a direct action of the solvent, as the quantity present was found in control experiments to be innocuous. Taken on the whole, the results indicate that eggs of *Hadena oleracea* are killed by concentrations rather lower than will kill eggs of *S. tetralunaria*. The insect however could not be controlled in practice by the use of dinitro-cresol, because the eggs are normally laid on foliage, which would be injured by spraying with this substance.

Dinitro-cresol is slightly soluble in water, and a second series of experiments was carried out with saturated and half-saturated solutions of dinitro-cresol in distilled water, tap water and a 1 per cent. solution of saponin in water. Unfortunately, the eggs available were mostly laid rather later than those used in the other experiments, and the control batches gave such irregular figures for the percentage hatched that little value can be attached to the results.

*Eggs of the Magpie Moth (Abraxas grossulariata L.).*

The number of Magpie moth eggs available was small, for the moths did not lay well in captivity; but results in close agreement have been obtained with dinitro-cresol in two successive years. The figures for the second series of experiments are given in Table III.

The eggs were sprayed on June 24 under the usual conditions. Hatching began on July 4 and continued until July 14. With eggs of this species, dinitro-cresol did not completely prevent hatching at any concentration tested below 0.5 per cent., but at concentrations down to 0.1 per cent., nearly all the larvae which hatched died shortly after emergence. Larvae from unsprayed (control) eggs were all healthy. It appears that eggs of this species resist penetration by dinitro-cresol more strongly than many other insect eggs, but that, none the less, quite low concentrations are finally fatal. The lower percentage of viable eggs killed at 0.2 and 0.3 per cent. than at 0.1 per cent. (see Table III) may be due to less complete contact with the spray in the

Table III. *Toxicity of 3 : 5-dinitro-o-cresol to eggs of Abraxas grossulariata L.*

Substance	Concentration in gm. per 100 c.c.	No. of eggs sprayed	No. killed		% eggs and larvae killed	% viable eggs killed (allowing for control)
			Not hatched	Larvae died on emergence		
Dinitro-cresol	0.5	55	55	—	100.0	100.0
"	0.3	51	16	31	92.2	87.8
"	0.2	67	24	35	88.1	81.3
"	0.1	41	26	15	100.0	100.0
"	0.05	47	16	15	66.0	46.6
"	0.025	55	28	2	54.5	28.6
"	0.01	26	7	1	30.8	—
					% eggs not hatched	
Controls	—	226	82	—	36.3	—

former cases, for the eggs used in these two tests were laid on small twigs, whereas in the rest of the series, they were on muslin. A similar point was noted in connection with Winter moth eggs (p. 164).

#### *Eggs of the Vapourer Moth (Orgyia antiqua L.).*

Little difficulty was experienced in breeding the Vapourer moth in considerable numbers and many thousands of eggs were obtained. It was found important to protect carefully from egg-parasites, for a high proportion of eggs collected in the field are parasitised by a Scelionid, *Telenomus dalmani* Ratz.

A series of experiments on these eggs was made with dinitro-cresol and sodium dinitro-cresylate at concentrations ranging from 0.5 to 0.05 per cent. and with a commercial tar-distillate spray fluid with known ovicidal properties at concentrations between 10.0 and 2.5 per cent. A single female Vapourer moth lays a batch of several hundred eggs and a portion (approximately half) of each batch used for a test was kept untreated as a control, thus providing a separate control figure for each test. This precaution seemed advisable in view of the wide range in the percentage of eggs which hatched normally in different batches. The eggs used in the experiments (some 9000 in all) were laid in August and September, 1925; they were sprayed on March 15, 1926, under the usual standard conditions; hatching began on June 5 and continued until the first week in July.

It is not necessary to give the results in detail, since at all concentrations above 0.05 per cent. dinitro-cresol and the sodium salt killed

100 per cent. of the viable eggs. At 0.05 per cent., dinitro-cresol killed 90.3 per cent., and the sodium salt, 73.1 per cent.; most of the unhatched eggs contained fully developed larvae which in many instances had bitten a hole in the shell but failed to emerge completely. The tar-distillate wash also killed 100 per cent. when used at 5 per cent. and above; at 2.5 per cent., most of the viable eggs contained fully developed larvae, many nearly out, but only about 10 per cent. emerged successfully.

It may be concluded that dinitro-cresol and the sodium salt are toxic to eggs of the Vapourer moth at a concentration of 0.1 per cent., and the tar-distillate fluid is toxic between 5.0 and 2.5 per cent.

Goodwin, Massee and Le Pelley<sup>(5)</sup> have recently published an account of the effectiveness of some tar-distillate washes under different conditions, which includes a series of experiments on eggs of the Vapourer moth with a "standard" tar-distillate preparation made from anthracene or green oil (sp. gr. 1.1), emulsified by means of rosin and castor oil soap. Their tests with this fluid at different concentrations show rather a lower toxicity than was found for the commercial tar-distillate referred to above. These authors also note that a proportion of the larvae which develop fail to emerge completely and they find that the percentage of these larvae "dead before total emergence" rises with the concentration of the spray fluid parallel with the rise in the percentage of viable eggs killed.

### *Eggs of Tortrix pronubana* Hb.<sup>1</sup>

This small moth was bred from larvae collected in the Royal Botanic Gardens, Kew, by permission of the Curator, on various plants in the glasshouses and on a bay hedge out-of-doors. The eggs are laid in masses and slightly overlap one another; each mass, which may contain any number up to 200 eggs or occasionally more, is covered completely by a transparent layer of mucilaginous material which hardens into a protective coating. The female moths normally oviposit on the upper surface of leaves, but will also readily lay on glass. The eggs themselves are semi-transparent and the development of the larvae can be followed; by inducing the moths to lay on microscope slides, convenient material for observation purposes was obtained. The percentage of eggs which hatched from fertilised egg-masses on glass was however rather irregular; in many cases, 50 per cent. or more failed to hatch, although the larvae, almost fully developed, could be seen inside the eggs.

<sup>1</sup> For details of the life-history and habits of *T. pronubana* see R. C. Fisher, *Ann. App. Biol.* 1924, 11, 395.

A number of experiments were made with dinitro-cresol and the sodium salt on eggs of this species. Detailed figures are not given, for at all concentrations down to 0.025 per cent., these substances proved toxic; the eggs were apparently less resistant than those of any other species tested.

Some evidence has been obtained with the eggs of various insects that there is a greater resistance to the toxic action of dinitro-cresol when the eggs are at an advanced stage of development. The eggs of *T. pronubana* appeared to afford suitable material for investigating this point. Batches of eggs at various stages of development, ranging from a few days after laying to a few days before hatching, were treated with dinitro-cresol at concentrations of 0.1, 0.05 and 0.025 per cent. In all cases, even at the lowest concentration, 100 per cent. of the viable eggs were killed; no increased resistance at the later stages was observed. It is possible that differences would have been noted if still lower concentrations had been used, but the limit of toxicity of dinitro-cresol to these eggs was not known at the time the experiments were made. Observations of the stage of development reached at the time of spraying showed, however, that in most cases little further development took place; penetration and toxic action were apparently rapid. With eggs in an early stage of development, disorganisation and discoloration of the contents was apparent a day or two after spraying, but this was less marked in the case of more developed eggs.

#### *Eggs of Red Spider.*

Laboratory experiments on the effect of dinitro-cresol and the sodium salt on eggs of the red spider laid on small branches of plum and apple trees were inconclusive. There was evidence of toxicity at concentrations of 0.5 and 0.25 per cent., but the numbers which hatched in the controls were very irregular and uncertain. No quantitative data have been obtained in field experiments, but general observations during two years indicated (2) that red-spider eggs are not killed by dinitro-cresol at 0.25 per cent. It is hoped to obtain more detailed information on this point.

#### *Effect on the Toxicity of Dinitro-cresol of washing Eggs with Water after Spraying.*

From the practical point of view, it is important to know whether rain falling within a short time after spraying affects the toxicity of dinitro-cresol or the sodium salt. The results of some laboratory experiments on this point with eggs of *Selenia tetralunaria* and *Hadena oleracea* are given in Table IV. Batches of sprayed eggs were thoroughly washed

with running water at various times after spraying and the percentage hatched compared with that from sprayed eggs receiving no further treatment. The general procedure was otherwise the same as in the experiments already described. Only the final figures for the percentage of viable eggs killed are given in the table; the average number of eggs used in each test was about 120 with *H. oleracea* but only about 30 with *S. tetralunaria*.

Table IV. *Effect on toxicity of dinitro-cresol and the sodium salt of washing eggs with water after spraying.*

		% viable eggs killed (allowing for control).				
<i>Selenia tetralunaria.</i>		Washed immediately after spraying without allowing to dry	Allowed to dry and then immediately washed	Washed 24 hr. after spraying	Washed 48 hr. after spraying	No treatment after spraying
	%					
Dinitro-cresol:	0.25	18.4	100	100	100	100
"	0.1	12.0	60	—	—	90-100
Sod. dinitro-cresylate:	0.25	10.9	0	100	100	100

(An average of 8% of the eggs in controls did not hatch.)

*Hadena oleracea.*

		No treatment after spraying	Washed 4 hr. after spraying
	%		
Dinitro-cresol	0.2	100	100*
"	0.1	100	100*
"	0.05	100	48.7*
"	0.025	41.1	(62.5)†
"	0.01	0	0
Dinitro-cresol ("emulsified disinfectant" as solvent)	0.2	100	100
"	0.1	100	100
"	0.05	100	0
"	0.025	80.2	20.9
"	0.01	21.9	0
Sod. dinitro-cresylate	0.2	100	75.2
"	0.1	100	9.0
"	0.05	100	(100)†
"	0.025	9	0
"	0.01	25	0

(An average of 9.2% of the eggs in controls did not hatch.)

\* Some of the eggs hatched but larvae died on emergence.

† The eggs contained developed larvae which failed to emerge.

The figures show that at concentrations of 0.1 per cent. or above, the toxicity of dinitro-cresol is little reduced by the washing with water so long as the liquid is allowed to dry on the eggs before washing takes place. This suggests either very rapid penetration or good adhesive properties. At concentrations below 0.1 per cent., the toxic effect is much reduced

by washing. Sodium dinitro-cresylate, as would be expected from its greater solubility in water, is more affected by washing after spraying than dinitro-cresol; at 0.25 per cent., the toxicity is appreciably lessened by washing 4 hours after spraying, but no effect is observed 24 hours after spraying. At lower concentrations, the effect is still more marked. If the eggs are washed immediately after spraying, without allowing the liquid to dry, little toxic effect is shown by either dinitro-cresol or the sodium salt at any concentration tested.

The field experiments discussed in this and a previous paper afford further evidence with regard to the performance of these substances under adverse weather conditions.

#### FIELD EXPERIMENTS.

In a previous paper (*loc. cit.*), experiments are described in which spray fluids containing dinitro-cresol or the sodium salt showed a high efficiency against eggs of the Hop-Damson Aphis on plum trees under field conditions. A quantitative method for judging the results was used. Further experiments under field conditions have been made, mainly on apples and black currants, and a short account of them is presented here.

##### I. At Woburn Fruit Farm, Ridgmont.

By kind permission of Messrs E. and G. Hobbs, experiments were made at Woburn Fruit Farm, with spray fluids containing (a) dinitro-cresol (D.N.C.), and (b) dinitro-cresol converted into the sodium salt by addition of the appropriate quantity of sodium carbonate (Na D.N.C.). An "emulsified disinfectant" was used as solvent for the dinitro-cresol, a 10 per cent. stock solution being prepared. The spray fluid as applied contained 0.25 per cent. dinitro-cresol and 2.5 per cent. of the solvent. For the sodium dinitro-cresylate spray fluid, a 4 per cent. stock solution in water was prepared, and the spray fluid as applied contained 0.25 per cent. dinitro-cresol in the form of sodium dinitro-cresylate and 0.25 per cent. soft soap to assist spreading and wetting. Eight Gascoyne apple trees and four Bramley Seedling trees were sprayed with D.N.C.; and twelve Gascoynes and four Bramley Seedlings with Na D.N.C. All were rather large bush trees. The spraying was carried out by Messrs Hobbs with a hand spraying machine worked at a nominal pressure of 120 lb.; the fluids appeared to spread and wet well and no special difficulties were met with.

The sprays were applied rather late in the winter, the D.N.C. on February 17 and the Na D.N.C. on February 18, 1926; at this date the

Gascoyne trees were still in a quite dormant state, but many buds on the Bramleys were showing signs of growing out. In each case, unsprayed trees in adjacent rows of the same block, of the same age and grown under the same conditions, served as controls. There was practically no drifting of spray on to the control trees. No separate control experiments were made with the "emulsified disinfectant" used as a solvent

Table V. *Field experiments on apples.*

Treatment	Variety	Tree no.	Insects found in 25 buds per tree					Total insects
			<i>Aphis pomi</i>	<i>Anur-aphis roseus</i>	<i>Psylla mali</i>	Capsids	Caterpillar	
Dinitro-cresol 0.25%	Bramley seedling	1.	—	—	—	3	6	9
		2.	—	—	—	—	4	4
		3.	—	—	1	—	3	4
		4.	1	—	—	—	6	7
		Totals	1	—	1	3	19	24
Controls	Bramley seedling	5.	9	1	13	3	28	54
		6.	4	—	70	2	23	99
		7.	1	—	44	—	18	63
		8.	36	32	34	6	20	128
		Totals	50	33	161	11	89	344
Dinitro-cresol 0.25%	Gascoyne	1.	—	—	—	1	—	1
		2.	—	—	1	2	1	4
		3.	—	—	3	3	—	6
		4.	—	—	—	1	3	4
		5.	—	—	—	1	—	1
		6.	—	—	—	6	1	7
		7.	—	—	1	4	—	5
		8.	—	—	—	1	6	7
		Totals	—	—	5	19	11	35
Controls	Gascoyne	9.	2	—	128	5	7	142
		10.	1	—	148	—	6	155
		11.	1	—	239	—	6	246
		12.	42	8	212	6	5	273
		13.	1	—	209	2	6	218
		14.	3	2	212	2	17	236
		15.	—	—	89	—	5	94
		16.	16	—	281	8	20	315
		Totals	66	10	1518	23	72	1679
Sod. dinitro-cresylate, equivalent to 0.25% dinitro-cresol	Bramley seedling	1.	—	—	1	1	2	4
		2.	—	—	—	2	1	3
		3.	—	—	3	—	10	13
		4.	—	3	4	1	5	13
		Totals	—	3	8	4	18	33
Controls	Bramley seedling	5.	8	10	130	—	31	179
		6.	—	4	124	2	16	146
		7.	—	1	151	—	10	162
		8.	—	4	128	1	20	153
		Totals	8	19	533	3	77	640



Table V (contd.).

Treatment	Variety	Tree no.	Insects found in 20 buds per tree					Total insects
			<i>Aphis pomi</i>	<i>Anur- aphis roseus</i>	<i>Psylla mali</i>	Capsids	Cater- pillar	
Sod. dinitro-cresylate, equivalent to 0.25% dinitro-cresol	Gascoyne	1.	—	—	2	—	1	3
		2.	—	—	2	—	3	5
		3.	—	—	2	1	—	3
		4.	—	—	—	—	2	2
		5.	—	—	—	1	2	3
		6.	—	—	18	3	2	23
		7.	—	—	8	—	1	9
		8.	—	—	2	5	—	7
		9.	—	—	3	2	—	5
		10.	—	—	7	2	—	9
		11.	—	—	4	—	2	6
		12.	—	—	8	1	—	9
	Totals	—	—	56	15	13	84	
Controls	Gascoyne	13.	2	—	84	1	9	96
		14.	11	1	178	1	3	194
		15.	—	—	164	1	3	168
		16.	3	1	198	3	4	209
		17.	23	—	229	—	7	259
		18.	3	—	297	1	7	308
		19.	2	1	384	3	19	409
		20.	8	3	178	1	7	197
		21.	1	1	346	1	21	370
		22.	1	—	71	2	8	82
		23.	4	6	375	3	11	399
		24.	—	—	152	2	6	160
	Totals	58	13	2656	19	105	2851	

for dinitro-cresol. Many laboratory experiments and the field experiments previously reported<sup>(1)</sup> have shown that, at the strength used, this material has no appreciable toxicity to most insect eggs.

On February 17, when the D.N.C. was applied, rain began about an hour after the spraying was finished and continued for the rest of the day, 0.25 in. of rain being recorded by the next morning. The Na D.N.C. was put on on the following day under excellent weather conditions and no rain fell for 24 hours after spraying.

It was not practicable to make any detailed counts of the insect eggs present on the trees before spraying, but a general survey showed the presence of great numbers of eggs of *Psylla mali* and smaller numbers of *Aphis* eggs, the former being more numerous on the Gascoynes and the latter on the Bramleys. Only a few eggs of Winter moth or other moths were noted, but, as will be seen later, there was actually a considerable infestation.

The trees were examined at intervals after the spraying. By the middle of April, hatching of *Psylla* and *Aphis* was judged to be complete,

nymphs (especially *Psylla*) were present in large numbers on the buds of the control trees, but few were noted on the sprayed trees. In order to obtain reliable figures by which to judge the results, a random sample of buds was picked—20 or 25 per tree—on April 19, from each of the sprayed trees and from the same number of control trees. The buds from each tree were placed in a separate receptacle and as soon as possible afterwards every bud was examined and the insects found counted and recorded. The figures obtained are summarised in Table V. Blossom buds were scarce on most of the trees, but where present, they were included in the samples as nearly as could be judged in the proportion in which they occurred on the tree.

The figures given in the table are the totals found in the samples of buds from each tree<sup>1</sup>. Comparison of the results from the sprayed trees with those from the controls shows that Green Apple Aphis (*Aphis pomi*), Rosy Apple Aphis (*Anuraphis roseus*) and *Psylla mali* were almost completely eliminated by the treatments. The number of caterpillars present was also greatly reduced; they were mainly Winter moth larvae with a small proportion of Tortricid larvae. A few capsid nymphs of a non-injurious species were found; there was no evidence that the treatments had affected their numbers. The two spray fluids—D.N.C. and Na D.N.C.—proved about equally efficient against eggs of Aphids, *Psyllae* and Winter moth; this constitutes a rather drastic test for the D.N.C. fluid in view of the long-continued rain shortly after spraying.

General observation of the trees entirely bore out the results from the counts; it was difficult to find any Aphis or *Psylla* nymphs on the sprayed trees and caterpillars were markedly less numerous than on the controls. *Psylla* nymphs could readily be found in numbers on almost every bud on the controls. No subsequent spray damage to the trees was noted throughout the season and the healthier foliage and absence of leaves curled by *Anuraphis roseus* was specially noticeable on the Bramleys. In spite of the great number of *Psyllae* on the unsprayed Gascoyne trees, there was little evidence of injury which could be attributed to their activities. The crop of apples was however exceedingly small on both varieties and no observations as to the effect of the treatments on the yield of fruit could be made. Both fluids had a marked general cleansing effect on the trees, mosses and lichens being killed. The sprayed trees could readily be picked out from neighbouring unsprayed trees by the appearance of the bark; and this difference in appearance was even more noticeable in the following winter.

<sup>1</sup> Where the number of *Psylla* nymphs present was great, the figures are approximate only.

On the whole the results confirm those already reported in regard to Hop-Damson Aphis eggs (*loc. cit.*). It may be added that when the experimental spraying with Na D.N.C. was finished, Mr Hobbs used what remained of the fluid in the machine for spraying a standard Rivers plum tree. The general Aphis attack on plums happened to be exceedingly bad but this tree remained almost unattacked throughout the season and its foliage was in marked contrast to that of neighbouring unsprayed trees of the same variety; it was the only tree that bore a crop of fruit.

## II. *At the Hertfordshire Farm Institute, St Albans.*

A small scale outdoor experiment with the dinitro-cresol spray fluids was made on black-currant bushes, kindly placed at our disposal by the Director of the Herts Farm Institute. The primary object was to ascertain whether serious damage to the bushes would result from delaying spraying until the buds had begun to grow out. One "Baldwin" and six "Seabrook" black-currants were sprayed on February 13, 1926 with the spray fluid containing 0.25 per cent. dinitro-cresol and 2.5 per cent. "emulsified disinfectant" as solvent; the same number were sprayed with the sodium dinitro-cresylate fluid at equivalent strength. The stage of development of the buds on the two varieties at the time of spraying is shown in the accompanying photograph (Fig. 1). The only injury to the bushes noted subsequently was a quite negligible yellowing of the extreme edges of a few of the first leaves on the "Baldwins." No trace of injury was found on the "Seabrooks." Although so late a date for winter spraying would naturally be avoided if possible, it is clear that no serious damage need be anticipated if rather late spraying is unavoidable.

A considerable number of Aphis eggs was present on the bushes; at the time of hatching, nymphs were abundant on neighbouring unsprayed bushes, whereas only an occasional insect could be found on those which had been sprayed. Later in the season, a severe attack of Aphis developed on the unsprayed bushes, the leaves of almost every shoot being badly curled. On May 13, the number of the terminals on last year's wood on which the upper leaves were (a) infested with Aphis and badly curled, and (b) free from Aphis and healthy, were counted on the sprayed bushes and on unsprayed bushes in the next row.

The figures give a rough estimate of the effect of the treatment:

	No. of shoots infested	No. of shoots healthy
6 bushes sprayed D.N.C.	1	35
6 controls	43	2
6 bushes Na D.N.C.	5	49
6 controls	39	3



Fig. 1. Showing stage of growth of black currants when sprayed.  
February 13, 1926.

A. "Baldwin."      B. "Seabrook."

Winged forms were present in abundance and secondary infection was taking place but the sprayed bushes remained much cleaner throughout the season and made better growth than those unsprayed; they also held their leaves longer.

A larger scale experiment on black currants is planned.

Our sincere thanks are due to Messrs E. and G. Hobbs, of the Woburn Fruit Farm, and to Mr C. E. Hudson, of the Herts Farm Institute, for giving us every facility and their personal assistance in carrying out the field experiments. We are also indebted to Mr J. C. F. Fryer, who kindly visited both experiments and independently confirmed the general observations recorded.

#### CONCLUSIONS.

The results of the laboratory and field experiments demonstrate the high toxicity of dinitro-cresol and the sodium salt at concentrations between 0.1 and 0.25 per cent. to the eggs of several species of insects; they also demonstrate the practicability of using these substances as winter spray fluids on dormant trees and bushes under field conditions. There appears to be little difference in the intensity of the toxic action of dinitro-cresol and of the sodium salt, but there is some evidence that the latter, owing to its higher solubility in water, may be more readily washed off if rain falls shortly after spraying.

No attempt has been made to go into the question of the best method for making up the materials in a form suitable for distribution but this should not present great difficulty. The cost per gallon of the spray fluids as used in the field experiments compared favourably with the cost of winter washes in general use.

The work reported here has been confined to experiments with dinitro-cresol and sodium dinitro-cresylate; it is probable that similar results would be given by 2 : 4-dinitro-phenol and its sodium salt. In the earlier laboratory experiments, 2 : 4-dinitro-phenol was shown to be only slightly less toxic to insects than 3 : 5-dinitro-*o*-cresol(2); the latter was chosen for investigations having a direct practical bearing, partly because it was the most toxic compound and partly because it was more easily worked up than dinitro-phenol into a form suitable for spraying.

The sodium salt of dinitro-phenol is known to be highly toxic to wood-infesting fungi of the "dryrot" type and is employed to some extent in practice to control these organisms. Our attention has been drawn

recently to experiments some years ago by Falck<sup>(6)</sup> on the toxicity of a number of nitro-derivatives of phenol to wood-infesting fungi. He included dinitro-*o*-cresol and its sodium salt in his tests and found the latter rather less toxic to the mycelium of these fungi than the sodium salt of dinitro-phenol but equally effective in inhibiting germination of spores.

Dinitro-cresol thus has fungicidal as well as insecticidal properties.

#### SUMMARY.

1. The toxicity of 3 : 5-dinitro-*o*-cresol and its sodium salt to the eggs of several species of moths has been determined under laboratory conditions. Both substances are toxic to eggs of the species tested at concentrations varying from 0.1 to 0.025 per cent.

2. With eggs of some insects, hatching is not entirely prevented by the action of low concentrations of dinitro-cresol and sodium dinitro-cresylate, but the majority of the larvae which emerge succumb within a few hours.

3. The eggs of "red spider" are very resistant to the action of dinitro-cresol.

4. At equivalent concentrations, dinitro-cresol and sodium dinitro-cresylate have approximately the same toxicity to insect eggs.

5. Washing eggs with water after spraying has no appreciable effect on the toxicity of dinitro-cresol, if the liquid is first allowed to dry on the eggs. Sodium dinitro-cresylate is more affected by washing after spraying.

6. Field experiments on apples and black currants with spray fluids containing dinitro-cresol at a concentration of 0.25 per cent. and sodium dinitro-cresylate at equivalent concentration showed that both materials were completely effective against *Psylla* and *Aphis* eggs and greatly reduced the numbers of caterpillars. There was no evidence of any effect on Capsid eggs.

7. Both fluids had a cleansing effect on the bark of the trees, killing algae, lichens, etc.; they caused no injury to the trees themselves. The results demonstrate the practicability of using dinitro-cresol and sodium dinitro-cresylate as winter spray fluids on dormant trees and bushes under field conditions.

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# THE RELATIONSHIP BETWEEN THE CHEMICAL CONSTITUTION OF ORGANIC COMPOUNDS AND THEIR TOXICITY TO INSECTS<sup>1</sup>.

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(With Two Text-figures and One Diagram.)

## INTRODUCTION.

INSECTICIDES are usually classed in two main groups: (a) Contact Insecticides, (b) Stomach Poisons. This classification has been criticised by Janisch<sup>(1)</sup> who, while admitting its practical convenience, considers it unsound on physiological grounds, his view being that there is substantially little difference in the physiological action between stomach, contact and respiratory poisons, death or narcosis being mainly due to an alteration of the colloidal condition of the protoplasts of the cell. The division is, indeed, arbitrary, as many insect poisons come in both of these groups, *e.g.* nicotine, which is a stomach poison and a contact insecticide. The term stomach poison is self-explanatory; any compound which, on ingestion in the intestinal tract of the insect, causes death is classed under this heading; but the phrase "contact insecticide" has only become standardised by common usage over a long period of time, and is used now only for want of a better term. It does not imply that any insecticide may act at a distance, but only that certain chemical compounds used in special classes of work are brought into external contact with the insect, whereas a stomach poison is sprayed upon foliage or placed in material used by the insect as food. Contact insecticides are employed against every class of insect, but particularly for the control of those which suck the juices of plants, and against insect eggs; the application of stomach poisons is limited to the control of biting insects.

Contact insecticides are used either as vapours or as sprays. The former, known as respiratory insecticides, find an application in glass-houses, closed rooms and for soil fumigation. Certain compounds, even when sprayed, are said to act chiefly by gaining access in the vapour phase to vital parts of the insects, and McIndoo<sup>(2)</sup> claims that nicotine when sprayed penetrates into the tracheal system in this way. Hydrocyanic acid, tetrachlorethane, chloropicrin, naphthalene have found a usage as contact insecticides almost entirely as fumigants, nicotine has

<sup>1</sup> Thesis approved for the Degree of Doctor of Science in the University of London.



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been widely employed both as a fumigant and for spraying purposes, but some of the plant poisons such as tubatoxin, a non-volatile solid, have so far only been used dispersed in spray fluids.

### *Insecticidal action in the vapour phase.*

Certain compounds have been employed for a considerable period of time as fumigants against insect pests. Hydrocyanic acid has been extensively employed in this way in America against scale insects, and for the control of white fly in glasshouses in this country. Tetrachloroethane and nicotine have also been employed in a similar way in glasshouses, chloropicrin in flour mills, and many attempts have been made with varying degrees of success to use volatile organic derivatives, such as carbon bisulphide, chloropicrin and naphthalene, as fumigants for controlling soil pests.

The first attempt to correlate the insecticidal values of organic compounds in the vapour phase with their physical properties was made by Holt(3), who concluded that the toxicity of volatile organic compounds to the cockroach increases with the boiling point, although compounds boiling over a certain critical temperature show a decline in toxic properties. This view was largely substantiated by the investigations of Moore(4) who, working with the house fly, concluded that: (1) the toxicity of a volatile organic compound is correlated closely with its volatility, decreasing volatility being accompanied by increased toxicity; (2) the boiling point is a general index of volatility; (3) compounds boiling at 225–250° C. are usually so slightly volatile that they kill only after long exposure.

In a paper on the "Influence of Chemical Constitution on the Toxicity of Organic Compounds to Wireworms" by Tattersfield and Roberts(5) these views are examined. The vapours of a large number of chemical compounds were tested, and it was found that within clearly defined chemical groups toxicity increases with the molecular weight thus, for example, among the hydrocarbons the order runs:

Pentane < hexane < heptane, benzene < toluene < xylene;

but in a series of similar compounds, for the same time of exposure, compounds above a certain molecular weight are not toxic. The correlation of toxicity with molecular weight, however, is by no means good when compounds belonging to different groups or, in certain cases, when compounds of the same group are compared. Thus the aliphatic amines as vapours show the same order of toxicity, and among the aromatic

amines it is a matter of importance whether the  $\text{CH}_3$  group is substituted in the ring or in the amino group. It is obvious, moreover, that vapour pressure is the factor limiting toxicity amongst the compounds of high molecular weight, their concentration in the vapour phase never reaching a value great enough to prove lethal in the time allowed.

The correlation of toxicity with the volatility or the vapour pressure of compounds is somewhat closer than that obtained for molecular weight; nevertheless, chemical constitution plays so important a part in determining toxicity as to render this relationship only roughly true within certain groups; compounds having vapours of an irritant nature, such as chloropicrin or ammonia, are highly toxic despite their high vapour pressures. Within certain chemical groups the relationship between evaporation rates and insecticidal action holds fairly closely, and the view expressed by Holt and Moore (*loc. cit.*) seems to be true within certain limits. It would appear, however, that this relationship is merely an indication that some property, other than volatility, but closely linked up with it, plays a part in toxic action. It is known that adsorption on a carbon surface increases with the boiling point of the adsorbed gas; if this be true for the more chemically inert vapours, say, benzene, toluene, etc., it might account for increased toxicity shown as the series is ascended, for it is reasonable to suppose that compounds which can be readily adsorbed along the tracheal system of an insect would be more effective than those less readily adsorbed. Moreover, the highly volatile but readily adsorbed ammonia gas is fairly highly toxic, and the fact that the amino and hydroxyl group greatly increase toxicity, when substituted in the benzene ring, is more satisfactorily accounted for on the supposition that both chemical reactivity and adsorption are increased by the process, than by a mere reference to a correlative lowering of the vapour pressure.

The category in which compounds of low volatility are placed is affected by the time during which the insect is exposed to the vapour. Naphthalene vapour is not toxic to wireworms in a period of 1000 minutes, but later experiments than those described in the above paper, in which naphthalene was mixed with soil infested with wireworms, show that it is highly poisonous if an exposure of several days is allowed.

The categories in which the compounds are placed will also depend upon the degree of resistance of the insect. For example, for a highly resistant insect a compound of low vapour pressure will have so small a concentration in the vapour phase as to be ineffective, whereas to a very vulnerable insect it might prove highly toxic.

Some compounds are specific in their toxic action. Pyridine in the vapour phase is unexpectedly toxic to both adult mites and their eggs<sup>(6)</sup>, small doses rapidly killing the adults, and a rather longer exposure at room temperature being completely effective against their eggs. Several investigators have shown that, in general, this compound has little or no insecticidal action, whether applied as vapour or in the form of spray, and its toxicity must therefore be regarded as specific to these organisms.

#### SPRAY FLUIDS.

##### *Laboratory methods for testing insecticide sprays.*

1. *Dipping method*<sup>1</sup>. This method was employed by Fryer, Stenton, Tattersfield and Roach rather extensively in the investigation of the insecticidal properties of *Derris* (7).

The plant extract or the chemical compound to be tested is suspended, in as fine state of division as possible, in a solution of saponin and water. The insects are completely immersed in 50 c.c. of the solution, five at a time for a period of 10 seconds. They are then rapidly transferred to an open vessel, at the bottom of which a piece of filter paper is placed to absorb the excess of the fluid, and the appropriate food supply of the insect is then put in the vessel. After immersion the insects are examined over a period of several days and finally tabulated under four categories: (1) the killed, (2) the moribund, (3) the slightly affected, and (4) the unaffected. By giving marks to the first three categories it is possible to plot the concentration of poison against the toxicity. The method, however, only gives good results when skilfully employed. Constant conditions in each test are a *sine qua non*, and the insects *must* be of about the same size and age. It cannot be used for sucking insects and, generally, only caterpillars of a fairly large size are suitable test subjects.

2. *Spraying method*. Owing to the many objections which can be raised against a dipping method, a new one was sought for and an apparatus was designed by Tattersfield and Morris<sup>(8)</sup> to spray a known volume of fluid under constant pressure over a constant period of time upon insects placed at a fixed distance from the spray nozzle.

Adult wingless females of *Aphis rumicis*, of a definite size and age and raised each year from one fundatrix have been employed as test subjects. After spraying, the insects without further handling are placed in covered Petri dishes near their food supply and examined over a period of two days (or occasionally three), although, in general, a period

<sup>1</sup> A similar method has recently been employed by Richardson and Smith<sup>(9)</sup>.

of 24 hours is sufficient to judge of the effects produced. Ten insects at a time are sprayed with different concentrations of the compounds, dispersed in a solution of 1 per cent. saponin in water. The four categories mentioned above have been used for grading the results, but in the diagrams the concentrations of poison are plotted against the sum of the moribund and dead reckoned in percentages of the total number of insects used. It is possible that this latter procedure does not allow for the complete grading of toxic effects, but it enables one to compare with considerable exactitude the toxicities of different compounds and different concentrations of the same compound.

The apparatus can be used for testing the toxicities of compounds to the eggs of insects. Counted batches of eggs, laid either upon muslin or glass slides are placed in the apparatus and sprayed in the usual way. The eggs are afterwards allowed to dry, placed in tubes and set aside for a period sufficiently long to allow complete hatching in the controls. The number of viable eggs killed can then be plotted against the concentration of the compound. By noting the position of the curves upon the diagram, the relative toxicities of a number of compounds at different concentrations can be rapidly compared. The eggs of *Selenia tetralunaria* Hufn. have proved of considerable value; they can be obtained laid on muslin in great numbers, the unfertilised eggs are readily distinguishable and can be eliminated before spraying. The apparatus has proved highly successful for both classes of work.

#### DISCUSSION OF THE RESULTS.

For the purpose of discussion it is convenient to divide the substances tested into (a) plant products, (b) synthetic organic compounds. This classification is rather artificial and is only used for purposes of simplification. The two classes merge into each other, but, as some of the more recently employed plant products have not been fully investigated and their constitution is unknown, it is convenient to consider them together. A few alkaloids are also classed under the heading Plant Products because few of them afford opportunity of discussing the bearing of structure on insecticidal action. Nicotine is, however, dealt with under the heterocyclic compounds.

##### (a) *Plant products.*

The most interesting plant products showing insecticidal properties are those which over a long period have been employed by the natives of the tropics for purposes of catching fish. Plants showing

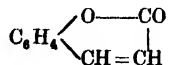
fish-poisoning properties belong to many natural orders, but it is an interesting fact that only those fish poisons derived from plants of the order Leguminosae have so far been found by us to have a high toxicity to insects. The best known of these plants is *Derris elliptica*, the 'tuba root' of the Malays, a plant employed sporadically in the East as an insecticide for a number of years. It has, however, only been used generally in England during a recent period; nevertheless, a considerable amount of work on its insecticidal properties has been done. It has been shown (10) that dry ether rapidly and completely extracts the poisonous principles and that the insecticidal value bears a direct relationship with the amount of the extract. The genuineness of the extract can be confirmed by determining the percentage content of methoxyl by means of the Zeisel method.

In *Derris elliptica* the characteristic poison to insects is a colourless crystalline substance—first isolated in a pure state by Ishikawa (11) in 1916—to which was given the name 'tubatoxin.' Tubatoxin is one of the most potent insecticides so far discovered, and is more widely distributed than was at first suspected. In addition to its occurrence as a constituent of *Derris*, it has been recently shown by us (12) to be present in quite considerable quantities in two species of plants of the genus *Lonchocarpus*, indigenous to British Guiana. They are known to the aborigines as 'white' and 'black haiari,' and are used for catching fish in a very similar way to the one in which *Derris* is employed by the Malays.

In *Derris*, tubatoxin is accompanied by a resin (derride) present in some samples in considerable quantities and showing insecticidal properties of a high order, whereas in the samples of haiari so far examined resinous material was present in smaller amounts and was found somewhat less toxic than tubatoxin. There is a considerable difficulty in freeing the resinous material from the last traces of tubatoxin, to which some of its toxic properties may be due. In addition to tubatoxin, a yellow crystalline derivative has been isolated from *Derris*; it was, however, subsequently noted that a series of yellow crystalline and non-toxic compounds could be prepared from tubatoxin by prolonged treatment with alcohol, and it is therefore highly probable that the yellow crystals were secondary products of the extraction process. Tubatoxin crystallises either in hexagonal plates or needles, it melts at 163.5° C. and the formula  $C_{18}H_{18}O_5$  has been ascribed to it by Ishikawa (*loc. cit.*) and by Kariyone and Atsumi (13), but  $C_{19}H_{18}O_5$  is considered as more probable by Takei (14). Takei states that 1.5 methoxy groups and one carbonyl group are present in the molecule, while saponification

with 3 per cent. hot alcoholic potash gives a phenol carboxylic acid of melting point 128–129° C.

It has been suggested by Priess<sup>(15)</sup> that a lactone structure might explain the toxic properties to fish of such compounds. The lactone coumarin



has been shown by Ellinger<sup>(16)</sup> to have definite narcotic properties, and some experiments on its insecticidal value showed that at a concentration of 0.25 per cent. it is almost completely toxic to *A. rumicis*. Too little, however, is known at present of the constitution of tubatoxin to make it more than a suggestion, that a lactone structure accounts for its toxic action—it is, moreover, many times more toxic to insects than coumarin.

In addition to *Derris*, the following plants used as fish poisons were also found toxic to *A. rumicis* (12, 17):

*Tephrosia Vogelii*—leaves, seeds and to a lesser extent stems and roots.

*Tephrosia toxicaria*—roots, stems; leaves not toxic.

*Black haiari*, genus *Lonchocarpus*—roots, stems; leaves not toxic.

*White haiari*, genus *Lonchocarpus*—roots, stems; leaves not toxic.

And more recently (data to be published separately):

*Tephrosia macropoda* (Native name iLozane) from South Africa—roots and stems.

*Tephrosia candida*, *T. Hookeriana* and *T. purpurea* have only inferior insecticidal properties, and no plant showing fish-poisoning properties, other than those belonging to the order Leguminosae, had any toxicity to insects in our experiments.

Hanriot<sup>(18)</sup> has ascribed the fish-poisoning properties of *T. Vogelii* to a crystalline product, 'tephrosine,' melting at 187° C. Two colourless crystalline derivatives, melting respectively at about 191° and 201° C., were isolated from the leaves of *T. Vogelii* but neither of them have toxic properties to insects commensurate with those shown by alcoholic extracts of the leaf<sup>(12)</sup>. A resinous product was, however, highly toxic, and it is possible that the drastic means of isolation recommended by Hanriot for the isolation of 'tephrosine' and the use of caustic soda to render soluble certain gums extracted from the leaf, had caused chemical change and that these crystalline compounds did not exist as such in the leaf. The crystalline derivative melting at 191° C., but not that melting at 201° C., gave the same colour reaction as tubatoxin when treated with nitric acid followed by ammonia, indicating a similarity of

structure. Hanriot ascribed to tephrosine the formula  $C_{31}H_{28}O_{10}$ , but nothing is known of its constitution.

A highly toxic resin was separated from the roots of *T. toxicaria*, in addition to certain non-toxic yellow needles, but no crystalline derivative showing toxic properties was isolated. The sample of root supplied to us was, however, too small to permit of an extended investigation upon this point. Further work is needed upon the derivatives found in the whole series of these plants, as they are of much interest, not only from a toxicological point of view, but also from that of the part played by them in the metabolism of the plant.

*The alkaloids*<sup>(12)</sup>. With the exception of nicotine, none of the alkaloids tested have any outstanding insecticidal properties. Eserine (physostigmine) is somewhat less toxic to insects than nicotine. Cytisine and lobeline, two alkaloids shown by Dale and Laidlaw<sup>(19)</sup> to have physiological reactions similar to nicotine are less toxic to insects than that alkaloid. It is of interest that an alkaloid so highly toxic to higher animals as coniine ( $\alpha$ -propyl-piperidine) has insecticidal properties of a distinctly low order.

(b) *Synthetic organic compounds.*

The following groups of compounds have been tested:

- (1) The aromatic hydrocarbons.
- (2) Certain chlorine derivatives of the aromatic hydrocarbons.
- (3) Certain nitro derivatives of the aromatic hydrocarbons.
- (4) Certain chlor-nitro derivatives of the aromatic hydrocarbons.
- (5) Certain hydroxyl derivatives of the aromatic hydrocarbons.
- (6) Certain nitro-hydroxyl and chlor-hydroxyl derivatives.
- (7) Certain aliphatic and aromatic amines.
- (8) Certain nitrogen heterocyclic compounds.
- (9) Certain fatty acids, their methyl esters, ammonium and sodium salts.

Many of these groups were tested upon both *A. rumicis* and the eggs of *Selenia tetrahunaria*.

(1) *Aromatic hydrocarbons*<sup>(20)</sup>.

When toxicities to *A. rumicis* of the compounds in these groups are compared the order runs:

Benzene < toluene < xylene < naphthalene > tetrahydro-  
naphthalene > decahydro-naphthalene<sup>1</sup>.

<sup>1</sup> The symbols <, > are employed to mean "less toxic than," and "more toxic than," respectively.

Up to naphthalene toxicity increases with molecular weight, but that chemical constitution plays a part is indicated by the reduction in toxicity resulting on the hydrogenation of the naphthalene molecule, until when a complete alicyclic structure is attained in decahydronaphthalene, the insecticidal properties become very small.

(2) *Chlorine derivatives of the aromatic hydrocarbons* (20).

The substitution of chlorine in the benzene ring intensifies the insecticidal action and with each successive chlorine atom, up to three, toxicity is increased. But later work has shown that further chlorination reduces toxicity, tetrachlor- and hexachlorbenzene having less effect than the trichlor derivative. Two noteworthy features of the group are (a) that it shows anaesthetic properties and (b) that *p*-dichlorbenzene is less toxic than the *o*-derivative. (Anaesthesia was demonstrated by the capacity of the insects to recover from a deeply moribund condition.)

The order of toxicity runs:

Benzene < chlorbenzene < *o*-dichlorbenzene < 1 : 2 : 4-trichlorbenzene > 1 : 2 : 4 : 5-tetrachlorbenzene.

$\alpha$ -Chlornaphthalene is more toxic than naphthalene.

(3) *Aromatic nitro compounds* (20).

The nitro group on substitution in benzene greatly increases toxicity to *A. rumicis*, the order running:

Benzene < nitrobenzene < meta-dinitrobenzene.

Trinitrobenzene is very difficult to suspend in a form suitable for spraying, and only on one occasion was it used, when it was sprayed on the eggs of the tomato moth (*Hadena oleracea*); it then proved less toxic than the dinitro derivative.

(4) *Aromatic chlor-nitro compounds* (20).

To *A. rumicis* the *o*-, *m*- and *p*-chlornitrobenzenes are all of the same order of toxicity as the 1 : 4-dichlor-2-nitrobenzene but rather less toxic, mole for mole than 1-chlor-2 : 4-dinitrobenzene. Picryl chloride rapidly decomposes on addition to water and its low toxicity is unquestionably due to this fact. The group presents no great points of interest, except the rather low toxic properties of such irritant compounds as the nitrobenzyl-chlorides.

(5) *Aromatic hydroxyl derivatives* (20).

There is little or no difference in the toxic values of phenol and the three cresols to *A. rumicis* or to the eggs of *S. tetralunaria*. Xylenol



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(a crude mixture of isomers) is more toxic to insects' eggs than either phenol or cresol. The effect of methylating the hydroxyl group of phenol is to reduce toxicity, anisole being distinctly less toxic than either phenol or its isomers the three cresols.

Table I. *The toxicities of the hydroxy- and methoxybenzenes to A. rumicis.*

(N = not affected, S = slightly affected, M = moribund, D = Dead.)







Compound	Concentration		N %	S %	M %	D %	M and D %
	Gm. per 100 c.c.	Mole per. 100 c.c.					
Phenol							
	5.0	.053	—	—	—	100	100
	3.5	.037	—	—	60	40	100
	2.0	.021	—	30	20	40	60
	1.0	.010	60	40	—	—	—
Pyrocatechol							
	5.0	.045	20	20	40	20	60
	3.5	.0315	40	50	10	—	10
	2.0	.018	80	20	—	—	—
Pyrogallol							
	15.0	.117	30	—	40	30	70
	10.0	.079	30	30	40	—	40
	7.5	.059	40	40	20	—	20
	5.0	.039	80	20	—	—	—
Anisole							
	10	.092	—	—	50	50	100
	7.5	.069	—	—	100	—	100
	5.0	.046	—	50	50	—	50
	3.5	.032	30	50	20	—	20
	2.0	.018	50	50	—	—	—
Veratrole							
	3.5	.025	—	—	—	100	100
	2.0	.014	—	—	—	100	100
	1.0	.007	—	60	40	—	40
	0.75	.005	70	20	10	—	10
Trimethoxybenzene							
1 : 2 : 3	2.0	.012	—	—	—	100	100
	1.0	.006	—	—	—	100	100
	0.75	.0045	20	—	10	70	80
	0.5	.003	60	20	20	—	20
	0.25	.0015	100	—	—	—	—

Table I gives the results obtained for phenol and anisole and certain polyhydroxyl and polymethoxyl derivatives of benzene. It will be seen from this table that toxicity declines as the number of hydroxyl groups is increased, the order running:

Phenol > pyrocatechol<sup>1</sup> > pyrogallol.

Although methylation of phenol reduces toxicity, the further in-

<sup>1</sup> The dihydroxybenzenes other than pyrocatechol were found less toxic than phenol.

crease in the number of methoxyl groups causes a rise in toxic values so that the order among the methoxyl derivatives is as follows:

Anisole < veratrole < trimethoxybenzene (1 : 2 : 3).

If the symbols > and < be taken as meaning "more toxic than" and "less toxic than," respectively, the diagrammatic arrangement found in Fig. 1 shows up a series of interesting relationships.

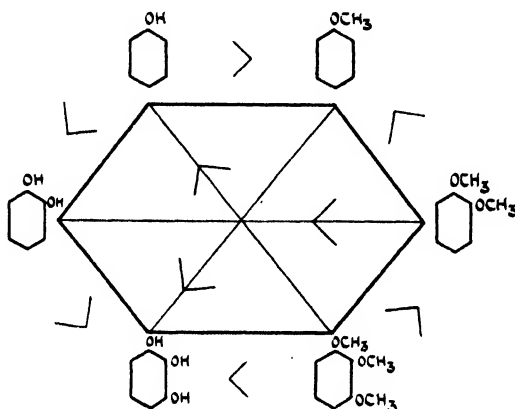


Fig 1. Showing relationships between the toxicities of the hydroxy- and methoxybenzenes.

The comparative toxicities obtained for the hydroxyl derivatives agree closely with those obtained by Cooper (21) for the germicidal power of these compounds. Both the insecticidal and antiseptic properties decline as hydroxyl groups are substituted in the phenol ring.

The introduction of hydroxyl groups in the phenol molecule increases solubility in water and decreases solubility in organic solvents. The partition coefficients of these compounds as between water and benzene were determined. The results obtained at 25° C. for the water/benzene ratio were as follows:

Phenol, 0.408; pyrocatechol, 9.174; pyrogallol, 125.

As the ratio solubility in water to that in benzene increases, there is a correlative decline in toxicity. The bearing of partition coefficients on toxicity is discussed more fully later, but that the relationship is not a simple one, and cannot be applied without reference to chemical constitution, is indicated by the fact that both methylation of phenol and the introduction of an additional hydroxyl group, which have opposite effects upon the solubility relationships, result in a decline in toxicity, and that while the progressive increase in the number of hydroxyl groups reduces,

the progressive increase of methoxyl groups increases toxic values. No suitable method for determining the partition coefficients of the methoxyl derivatives was found, one based on the Zeisel method yielding variable results. *Prima facie*, however, it would appear as if a correlation of the type found between solubility coefficients and toxicity among the hydroxyl derivatives would not hold for the methoxyl compounds.

(6) *Nitro-hydroxyl and chlor-hydroxyl derivatives.*

In Table II are set out the results of experiments on the toxicity of a number of simple nitro bodies to *A. rumicis* and certain insect eggs.

Table II. *Toxicities of various nitro compounds to A. rumicis and certain insect eggs.*

Compound	(Concentrations in mole per 100 c.c. giving 90-100 % control)		
	<i>A. rumicis</i> adult	<i>S. tetralunaria</i> egg	<i>Hadena Oleracea</i> egg
Benzene ... ..	Very slight	Not toxic	Not toxic
Nitrobenzene ... ..	·004-·006	>·04	·13
<i>m</i> -Dinitrobenzene ... ..	·0015	·03	·003
1 : 3 : 5-Trinitrobenzene	—	—	>·012
Phenol ... ..	·026-·037	>·05	—
<i>o</i> -Nitrophenol ... ..	·018	>·04	—
<i>m</i> -Nitrophenol ... ..	·007	>·015	—
<i>p</i> -Nitrophenol ... ..	·007	>·015	—
1 : 2 : 4-Dinitrophenol ...	·0013	·0027	·0005
1 : 2 : 5-Dinitrophenol ...	—	—	>·004
1 : 2 : 6-Dinitrophenol ...	—	—	·001 (approx.)
1 : 2 : 4 : 6-Trinitrophenol	·004	>·004	—
<i>o</i> -Cresol ... ..	·02	>·05	—
<i>m</i> -Cresol ... ..	·02	>·05	—
<i>p</i> -Cresol ... ..	·02	>·05	—
3-Nitro- <i>o</i> -cresol ... ..	>·013	>·013	—
5-Nitro- <i>o</i> -cresol ... ..	·0032	—	—
2-Nitro- <i>m</i> -cresol ... ..	·0065	>·013	—
4-Nitro- <i>m</i> -cresol ... ..	·013	>·013	—
6-Nitro- <i>m</i> -cresol ... ..	—	>·013	—
3-Nitro- <i>p</i> -cresol ... ..	·0065	>·013	—
3 : 5-Dinitro- <i>o</i> -cresol ...	·0005	·001	·00025
3 : 5-Dinitro- <i>p</i> -cresol ...	>·01	·005	—
Trinitro- <i>m</i> -cresol ... ..	·004	>·004	—
Anisole ... ..	·069	>·05	—
<i>o</i> -Nitro-anisole ... ..	·0065	>·013	·013
<i>p</i> -Nitro-anisole ... ..	·0065	>·013	·0065-·013
2 : 4-Dinitro-anisole ...	—	·01	·025
Trinitro-anisole ... ..	—	—	>·02

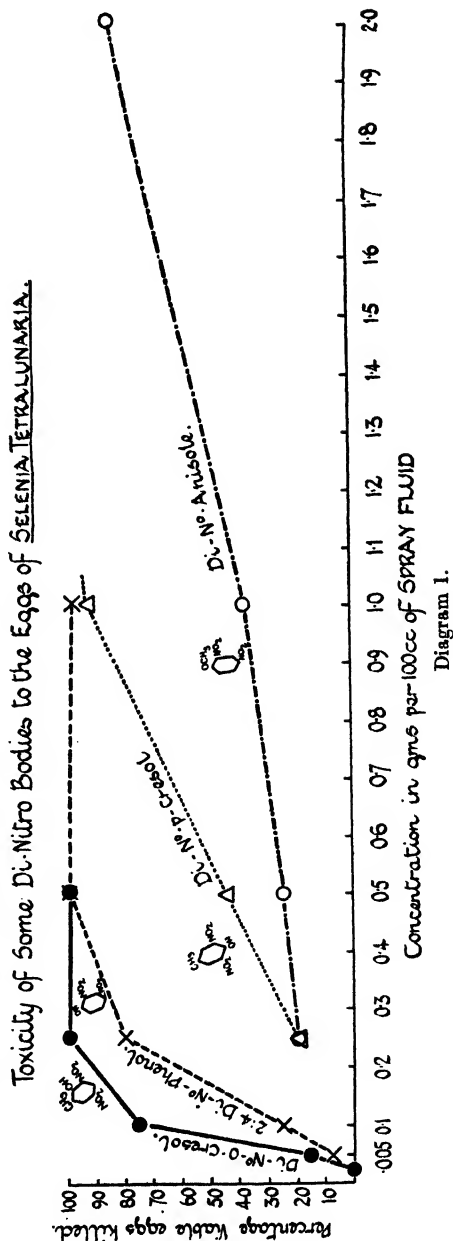
NOTE: To the eggs of *Cheimatobia brumata* and *Orygia antiqua* 3 : 5-dinitro-*o*-cresol was toxic at concentrations of ·0005 mole/100 c.c.

The substitution of one nitro group into the phenol molecule increases toxicity to *A. rumicis*, but not to any considerable extent; the para- and meta-derivatives are somewhat more toxic than the ortho-derivative. Very little difference is to be noted in the insecticidal values of the

mono-nitrocresols, either among themselves or when compared with nitrophenols. The *o*- and *p*-nitroanisoles are considerably more toxic than anisole but are only of the same order of toxicity as the mono-nitrophenols and cresols. Further nitration of the phenols and cresols to the dinitro bodies has a profound effect upon insecticidal values, the 2 : 4-dinitrophenol and 3 : 5-dinitro-*o*-cresol being highly toxic substances, both to *A. rumicis* and insect eggs. 3 : 5-Dinitro-*p*-cresol is much less toxic than either of these two compounds, as is also 2 : 4-dinitroanisole. Diagram 1, in which the concentration is plotted against the number of the viable eggs of *S. tetralunaria* killed, gives a conspectus of the toxic relationships of the dinitro derivatives.

The relative position of the two nitro groups modifies toxicity to insect eggs. When tried upon the eggs of the tomato moth (*Hadena oleracea*), the 2 : 4- and the 2 : 6-dinitro derivatives of phenol were lethal down to a concentration of 0.2 per cent. whereas the 2 : 5-compound was not completely toxic at 0.75-1.0 per cent.

Further nitration reduces toxicity, trinitrophenol (picric acid) and trinitro-*m*-cresol are less toxic to *A. rumicis* and the eggs of *S. tetralunaria* than the 2 : 4-dinitrophenol and 3 : 5-



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dinitro-*o*-cresol; and it has been shown in experiments with the eggs of the tomato moth that trinitro-anisole is less toxic than the di-derivative.

Plantefol (22) has shown for the nitrophenols that to *Aspergillus niger* the *o*-derivative was the least toxic and the *p*- the most toxic of the mono-derivatives, that 2 : 4-dinitrophenol was at least ten times as

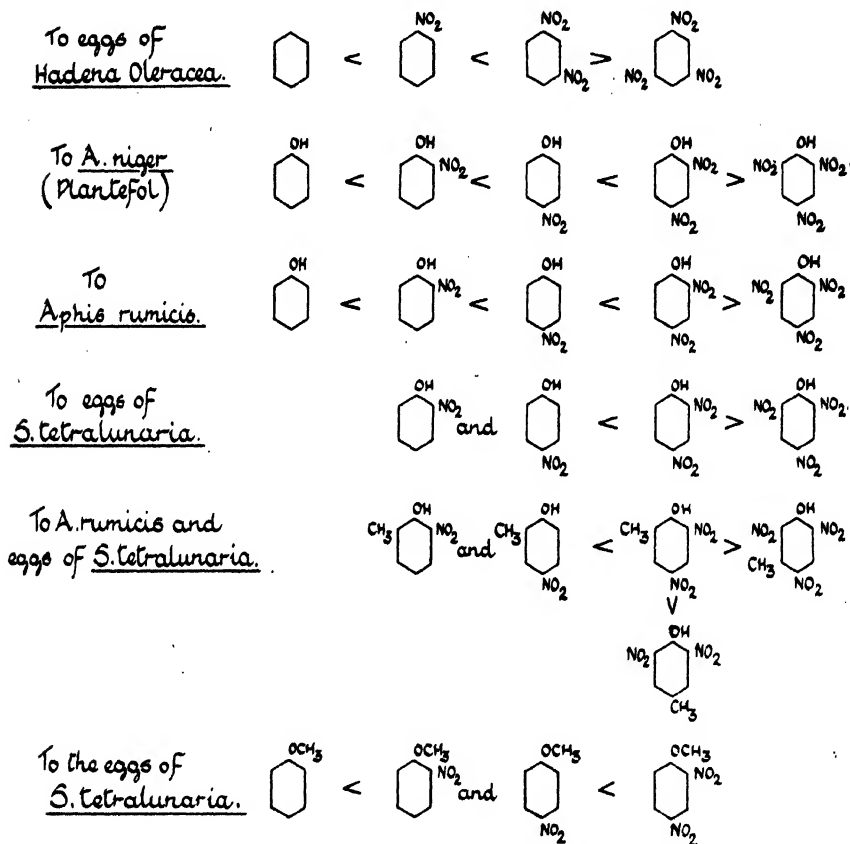


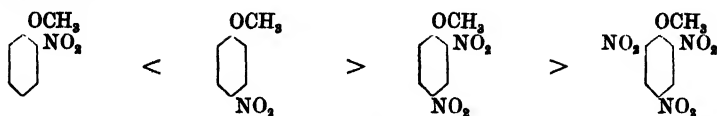
Fig 2. Showing toxic relationships of the nitro compounds to various organisms.

toxic as the mono-derivatives and that 2 : 4 : 6-trinitrophenol was of the same order of toxicity as meta-nitrophenol.

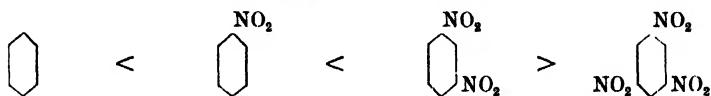
Using the symbols > and < in the way previously defined, these results may be expressed diagrammatically as in Fig. 2<sup>1</sup>. Recent work, however, on the toxicity of these and allied compounds to the eggs of

<sup>1</sup> The OH group is regarded as occupying the No. 1 position in Fig. 2, with the object of showing up relationships.

the tomato moth has indicated that for these eggs the order for the nitro-anisoles is:



indicating that the variety of the egg may have some effect upon the order, but it may be pointed out that for the last members of this series it is very difficult to obtain suspensions suitable for spraying, owing to their relatively low solubilities. It is of interest to compare the toxicities of the nitrobenzenes to these eggs, the order being:



It can therefore be stated that in general the dinitro derivatives of benzene and the phenols are more toxic than the corresponding mono-derivatives on the one hand and the tri-derivatives on the other.

The chlor-hydroxyl derivatives of the phenols do not present any points of great interest.

It is not easy to account for the differences in toxicity found among the nitro compounds, either on grounds of chemical constitution or physical properties. Sidgwick and his co-workers<sup>(23)</sup> have shown that 2 : 4- and 2 : 6-dinitrophenols are sharply differentiated from the other dinitrophenols except the 2 : 5-compound in their solubility in water. They are less soluble and their critical solution temperatures much higher. Their solubilities in benzene, however, do not appear to be anomalous and one would expect therefore their partition coefficients water/benzene to be low. But in these respects they are similar to *o*-nitrophenol, the toxicity of which is small, whereas these dinitrophenols, particularly the 1 : 2 : 4, have an insecticidal value of a high order. The difference in the solubility in water between the orthonitrophenol and its two isomers is so marked as to have given rise to the suggestion that it possesses a different structure from the meta- and para-; the same reasoning, however, indicates a similar constitution for 2 : 4-dinitrophenol as for *o*-nitrophenol.

The toxicity of 3 : 5-dinitro-*o*-cresol<sup>1</sup> was found to have such a high insecticidal value that an intensive study of its toxicity has been made both in the laboratory and the field<sup>(24)</sup>. The sodium salt is slightly

<sup>1</sup> The deleterious effect of this compound and its salts on foliage of all kinds rendered its use as a summer wash impossible. Main attention was, therefore, paid to its toxicity to insect eggs, as there seemed to be a likelihood of it being safe in use on dormant trees as a winter wash.

less toxic than dinitro-*o*-cresol but not to such a degree as to invalidate its use for field trials. Both of these compounds, tested on many varieties of insect eggs in the laboratory, have shown a high ovicidal value. This was amply confirmed in the field trials(24) which indicated that at concentration of 0.15–0.25 per cent., calculated as dinitrocresol, both these compounds are about equally effective. That both dinitrocresol and its sodium salt controlled a bad infestation of hop-damson aphids, when used as winter sprays upon the eggs of this insect, was confirmed by the fact that these trees were practically free from this pest during the following spring. More recent experiments(25) have shown that, used as a winter wash at these low dilutions, dinitrocresol and its sodium salt are able to control aphides on black currants, and were effective against *Psylla*, aphides, and winter moth upon apple. No damage was done to the trees.

(7) *The amines*<sup>1</sup>.

Among the aliphatic amines, the quaternary methyl- and ethylammonium compounds are of peculiar interest. Gram for gram and mole for mole tetramethylammonium is more toxic than tetraethylammonium, used either as bases or as salts. The toxicity of the salts of the tetramethylammonium base is surprisingly high. It has been shown by Dale(26) and his co-workers that tetramethylammonium, in contradistinction to tetraethylammonium, has in a marked degree certain physiological reactions characteristic of nicotine. Our experience has been that it is distinctly more rapid in its toxic action on insects than the ethyl compound. Dale found the property of paralysing ganglion cells to be as marked a characteristic of the tetraethylammonium as of tetramethyl derivative, which would indicate that the whole series of properties, in which the latter simulates nicotine, is of importance in determining its insecticidal value.

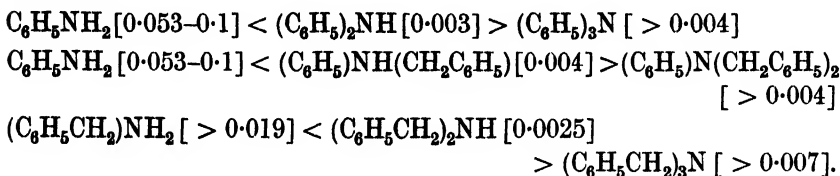
It is a matter of common knowledge that physiological differences do exist between methyl and ethyl alcohol and Flusin(27) has shown that they penetrate animal membranes at very different rates. Such differences in toxicity to insects as those observed for tetramethyl- and tetraethylammonium, compounds very similar in chemical constitution and physical properties, are, however, very difficult to understand, and in the present state of our knowledge, the view expressed by Dale(28) to explain their differences in physiological behaviour, that it is necessary

<sup>1</sup> A more detailed account of the toxic relationships of the amines will be published separately.

to postulate "some peculiar conformation of the reactive surfaces to which the methyl groups are adjusted which the ethyl groups will not fit," seems the only one capable of accounting for the differences between them in their toxicity to insects. So far, no group has been found which will replace with advantage the methyl radical in the quaternary ammonium compounds.

*The aromatic amines.* The introduction of the amine radical into benzene increases toxicity, and the substitution of aliphatic radicals, either into the ring or the amino group gives rise to a further increment, but not to any considerable degree. The introduction of an aromatic nucleus into the amine group of aniline increases toxicity to a marked extent; a second aromatic group, however, results in a loss of toxic properties.

The following sets of relationships were noted, the figures in square brackets representing the lethal concentrations in fractions of a mole per 100 c.c.



The residual hydrogen attached to the amino group of diphenylamine can be replaced by a methyl group without loss of toxic properties. (Methyldiphenylamine 0.002 mole/100 c.c.)

No attempt has been made to account for the fairly high toxic values of diphenylamine, benzyaniline and dibenzylamine when compared with the mono- and tri-derivatives. They run, however, closely parallel with those found for the nitrobenzenes and nitrophenols. It is perhaps suggestive that the amino group and imino groups are polar to water and that a light group such as methyl only modifies this property to a small extent, whereas a heavy molecule of the phenyl or benzyl type completely destroys it. Later work on the fatty acids indicates it to be probable that the water-solubility relationships may have some bearing on the toxicity of a compound.

$\alpha$ -Naphthylamine (0.007 mole/100 c.c.) is more toxic than aniline (0.053 mole/100 c.c.), but substitution in the amino group does not increase the toxicity of  $\alpha$ -naphthylamine to the same extent as it does that of aniline, *e.g.* mole for mole, phenyl- $\alpha$ -naphthylamine (0.0034 mole/100 c.c.) is about as toxic as diphenylamine (0.003 mole/100 c.c.).  $\alpha$ -Naphthylamine and its derivatives are more toxic than  $\beta$ -naph-



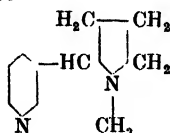
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thylamine and its derivatives. The most toxic amine derivatives tested were *o*-nitraniline (·0018--003 mole/100 c.c.) and a crude sample of nitro- $\alpha$ -naphthylamine.

### (8) *N-heterocyclic compounds*<sup>1</sup>.

Nicotine is the most highly toxic of all the heterocyclic compounds tested. It has been in use as a contact insecticide for many years, and is regarded as the standard material for this purpose. Our data show its high toxicity and that it is inferior only to tubatoxin and the compounds isolated from the leguminous plants used as fish poisons. In insects, as well as in the higher animals, it kills by paralysis of the central nervous system.

Nicotine has the structure



The two nuclei, pyridine and pyrrole, which are characteristic of this molecule, are only feebly toxic to insects. Hydrogenation of these two rings increases their toxicity; piperidine and pyrrolidine are distinctly more toxic than pyridine or pyrrole but they are much less so than nicotine itself. As it has been shown also that synthetic *d*-nicotine<sup>(29)</sup> is less toxic to the higher animals than the naturally-occurring laevo-compound, it would appear that the whole make-up of the nicotine molecule, including its spatial arrangement, contributes to its toxic properties. The order of toxicity among the simpler heterocyclic derivatives is as follows:

Pyrrole < pyridine < picoline < lutidine < quinoline and  
isoquinoline < acridine.

Benzyl-pyridine (a mixture of  $\alpha$  and  $\beta$  derivatives) has insecticidal properties of a fairly high order.

It can be deduced from our experiments that the toxicity of pyridine can be enhanced by hydrogenation, by the attachment of certain side chains, and groups of the benzyl type (probably preferably in the  $\beta$  position). Richardson and Smith<sup>(30, 8)</sup> have also shown that certain of the dipyridyls have insecticidal properties of a high order.

(9) *The fatty acids*<sup>2</sup>, etc. Siegler and Popenoe<sup>(31)</sup> have shown that certain of the fatty acids are highly toxic to aphids, capric and lauric acids being particularly potent. Their complete data have not been available,

<sup>1</sup> A more detailed account of the toxic relationships of the *N*-heterocyclic compounds will be published separately.

<sup>2</sup> A detailed account of the insecticidal properties of the fatty acids will be published separately.

but they state that for the acids tested, the intensity of the insecticidal action is greater, the greater the molecular weight up to a point in the series, after which it declines. A considerable number of the acids, their methyl esters and ammonium and sodium salts have been included in our survey, as they appear to offer facilities for studying change of toxicity with increasing molecular weight and the correlative changes in physical properties. Moreover, as the acids can be readily esterified and saponified it seems possible to determine with some accuracy the toxic significance of the carboxyl groups which remains constant and of the hydrocarbon chain which varies as the series is ascended. The salient data for this group are presented in Table III.

Table III. *Toxicities of the fatty acids, their methyl esters, and ammonium salts to A. rumicis.*

No. of carbon atoms in acid	Name of acid	Concentrations in mole/100 c.c. giving 100 % moribund and dead			Partition coefficients of acids at 25° C. water/olive oil	Surface tension of saturated solutions in water at 25° C. (Traube's Stalagmometer) Water taken as 71.8 dynes/cm.
		Acid	Ammonium salt	Methyl ester		
Saturated acids:						
1	Formic	·076	—	·08	10.5	—
2	Acetic	·083	—	Toxicity very slight	5.15	—
3	Propionic	·067	—	”	2.9	—
4	Butyric	·057	—	”	1.2	—
5	Valeric	·02	—	”	·38	—
6	Caproic	·009	·03	·115	·12	—
7	Oenanthic	·004	·015	·07	·03	—
8	Caprylic	·002--003	·007	·02	·01	—
9	Pelargonic	·001--0017	·002	·01	<·0025	—
10	Capric	·0005--001	·002	·003 (?)	<·001	32.64
11	Undecylic	·0005--0007	·0017	—	—	47.1
12	Lauric	·003	·0014	·01	Very small	70.1
13	Tridecylic	·0015--002	·002	—	”	—
14	Myristic	Toxicity very slight	·008	—	”	—
15	Isocetic	Not available	—	—	”	—
16	Palmitic	Toxicity very slight	—	—	”	71.2
18	Stearic	”	—	Toxicity very slight	”	—
Unsaturated acids:						
11	Undecenoic	·001	·001--003	·002	—	—
18	Oleic	·0028--0035	·0018	·012	Very small	71.3

NOTE: Ammonium hydroxide has a negligible toxicity.

#### *Tests upon A. rumicis.*

Formic acid and methyl formate prove exceptional; they are more toxic than acetic acid and methyl acetate respectively. Formic acid cannot

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be regarded as a typical fatty acid, but its exceptional toxicity indicates that considerations of chemical structure (in this case aldehydic) cannot be omitted in the discussion of the intensity of insecticidal action. The data presented show that as the series is ascended toxicity increases from acetic to undecylic acid and then declines somewhat at dodecylic (lauric) and tridecylic acids and disappears in the acids from tetradecylic (myristic) upwards.

The two unsaturated acids undecenoic and oleic were included in the tests. Undecenoic acid has a toxicity of the same order as undecylic, but oleic acid containing 18 carbon atoms is less toxic than undecenoic but distinctly more toxic than stearic acid.

Methylation of the carboxyl group reduces toxicity throughout the whole series. The sodium soaps of the acids, with the exception of sodium oleate, have also lower insecticidal values than their corresponding acids, but the effect of neutralisation with ammonia depends upon the molecular weight of the acid, for although among the lower acids it materially reduces toxicity, among the higher acids from nonylic to tridecylic its effect is small, and for myristic and oleic conversion to the ammonium salt enhances toxicity. It is obvious from this data that both the carboxyl group and the hydrocarbon chain are responsible for the determination of the insecticidal value of these acids, but the length of the hydrocarbon chain sets a limit upon toxicity. The character of the hydrocarbon chain is of importance as oleic, an unsaturated acid, is more toxic than stearic acid with the same number of carbon atoms but possessing a saturated chain.

### *The influence of molecular weight on the insecticidal values of the fatty acids, etc.*

The data presented in Table III indicate that, among these acids, toxicity increases with molar weight up to a certain limit, and then declines; but the correlation between the size of the molecule and toxicity is only partial, and its constitution plays a part in the effect; for example, formic acid is more toxic than acetic, methyl formate than the methyl esters of the next two or three higher acids; and oleic acid is more toxic than stearic. Moreover, in each of the series, the acids, esters and salts, toxicity declines when a certain molecular weight is reached, and when cross references are made from one series to another it is observed that the methyl esters may be less toxic than the acid of the same molecular weight. It would therefore appear as established that the toxicity of the acid is dependent partly on chemical constitution,

and partly on one or more physical properties colligative with the size of the hydrocarbon chain. A few of these physical properties are examined.

*Physical state.* The series of acids contains members some of which, at ordinary temperatures, are liquid and others solid, and it was considered that the physical state of the compound might have some bearing on toxicity. The tests upon *A. rumicis*, however, indicate that to this insect toxicity is not sharply defined by the solid or liquid state of the acid, the solid acids decylic and undecylic are more toxic than nonylic (liquid), and although oleic (liquid) is more toxic than stearic (solid) it is less so than decylic acid (solid).

A certain number of tests, in which the eggs of *S. tetralunaria* were used, demonstrated, however, that the physical state could not be discounted entirely. To these eggs a concentration of 2.0 per cent. of the different acids gave very variable results. It was found that whereas nonylic acid (pelargonic), a liquid, kills 80 per cent. of the viable eggs at this concentration, decylic acid (capric) melting at 31° C. is without toxic properties at this concentration (it is one of the most toxic of the acids to *A. rumicis*); the undecylic acid used was more toxic than decylic and had a melting-point of 27.5° C. but showed the property of super-cooling. The next two acids, dodecylic (lauric) M.P. 44° C. and tetradecylic (myristic) M.P. 54° C., are non-toxic at a concentration of 2 per cent., but the unsaturated acid undecenoic (M.P. 24.5° C.), a liquid at summer temperature, and the liquid acid oleic have definite toxic properties at this concentration, undecenoic (M.P. 24.5° C.) acid is more toxic than undecylic (M.P. 27.5° C.).

It is probable therefore that solid acids do not penetrate the shell of an egg as rapidly as liquid acids and that the dependence of toxic properties in this case on the physical state of the acid is real. There is, however, some probability that the factor involved is one of solubility, to be considered later.

*Volatility.* There are good grounds for believing that volatility will affect toxicity, for a highly volatile compound after spraying might evaporate from the surface of the insect and be dissipated before proving lethal. But that this property has only a bearing in extreme cases is shown by the fact that the fairly volatile acids of the series are more toxic than acids such as myristic and palmitic, the vapour pressures of which at ordinary temperatures are practically nil.

*Dissociation constants.* A number of observers have shown that mineral acids do not penetrate uninjured cell walls so readily as the fatty

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acids. Degree of dissociation is one of the physical factors which may determine such differences in penetrating powers. The toxicity data, however, do not indicate that the dissociation constants of the fatty acids have any important bearing upon their insecticidal values. Formic, a fairly strong acid, is not too markedly differentiated in its toxic properties from the rest of the series and from acetic to decylic there is a steady rise in toxicity, although the dissociation constants are all of the same order of magnitude.

*Partition coefficients.* The study of the allied subject of anaesthesia and narcosis has given rise to an extensive literature dealing with the chemical and physical properties which determine it. One of the most important hypotheses advanced is that associated with the names of Meyer and Overton, who independently expressed the view that a measure of narcotic action of a substance is given by the partition coefficients; that is, by the relative solubility of a substance in lipoid or oil to that in water. This theory has been subjected to much criticism and it has been pointed out that being in reality a theory of penetration, it only suggests that the narcotic action of a substance is in some way bound up with its interaction with cell membranes, of which lipoids and fats are regarded as constituents. Although the hypothesis is now regarded as too specific to account adequately for narcosis, and cannot be applied without reference to other physical properties exhibited by the narcotic, a number of investigators have taken the view that the penetration of the fatty acids may well be determined by it. As readiness of penetration is one of the prime factors affecting toxic action, the partition coefficients of the fatty acids between olive oil and water were determined.

In Table III the molar concentrations of the acids, giving 100 per cent. moribund and dead, are stated along with the ratios of their solubilities in water to that in oil, determined by extracting olive oil, containing a known amount of the fatty acid, with water at 25° C. Formic acid is exceptional, but from acetic to decylic acid there is a steady rise in toxicity with a decline in the value of this ratio. Higher than this the correlation fails. Oleic acid, for example, is less toxic than one would expect on the assumption that the correlation held. The theory therefore cannot be applied without consideration of other factors. If the relative solubility in fats and oils is a determining factor for high toxicity, it is reasonable to suggest that the absolute solubility in media of this kind, of which the partition coefficient tells us little, should play a part in the effect. The acids which are only slightly soluble in oil,

such as myristic, palmitic and stearic, show little if any toxic action; on the other hand, however, oleic acid, miscible in all proportions with oil and most organic solvents has a toxicity much less than would be expected from this assumption; and the question arises whether water solubility does not play a significant part in toxic action; for this group of acids in addition to the hydrocarbon chain, polar to oil, is characterised by a carboxyl group, polar to water. The fact that the ammonium salts of myristic and oleic acids, which are readily soluble in water, are more toxic than the corresponding acids may be suggestive that in some, as yet undetermined, way the toxicities of the fatty acids are bound up with the attraction of the carboxyl group to water, in addition to that exhibited for oil by the hydrocarbon chain.

The Partition Coefficient Theory in general does not differentiate clearly between the use of oil and lipoid as the organic solution medium. Nevertheless, the difference in chemical and physical properties shown by a typical glyceride and a true lipin like lecithin are so marked, as to render it highly probable that the partition coefficients should be determined for water and an organic solvent approximating more closely in physical properties to the constituents of the living membrane than does olive oil. Phillipson<sup>(32)</sup> found that the way in which his artificial membranes behaved, in their permeability to the acids, depended entirely upon whether or not they were impregnated with a *complete* etherial extract of muscle. A lipin like lecithin exhibits the property of imbibition of water, a property which sharply differentiates it from any true oil or fat.

The conclusion drawn from the data presented is, that although the theory in its simplest form does not afford a complete explanation of the toxicity of the fatty acids, and cannot be applied without reference to chemical constitution<sup>1</sup>, nevertheless the correlation between partition coefficients and the insecticidal values of many of the fatty acids is so close as to warrant further experimental work on the relationships existing between solubility, penetration and toxic action.

*Surface tension.* The researches of I. Traube<sup>(33)</sup> and of Czapek<sup>(34)</sup> have demonstrated the importance of the part played by surface tension phenomena in the penetration of cell membranes by chemical compounds. Czapek showed that for the fatty alcohols penetration of certain plant membranes took place when the surface tension of the alcoholic solutions reached a critical value of about 0.68 that of water. Traube demonstrated

<sup>1</sup> *E.g.* methylation of the carboxyl group which presumably decreases the values of the partition coefficients water/oil results in a general decline in toxic properties.

that lipid-free cells can be subjected to the action of narcotics, and a long series of papers by him since 1904 has brought into prominence the significance of surface tension in determining rate of penetration and osmosis. Traube has employed his 'Cohesion Pressure' (Haftdruck) theory to account for the action of drugs, antiseptics and narcotics. The cohesion pressure is related to surface tension in that the more a solute lowers the surface tension of a solvent the less its cohesion pressure in the solvent.

The surface tensions of the aqueous solutions of the fatty acids from formic to decylic have been worked out by Harkins, Clark and King, and very complete data has been published by King (35). Their data show that the relative lowering of the surface tension of water increases with the molecular weight of the acid, and there is, on the whole, a similarity between the adsorption curves for the fatty acids given by King, and curves relating toxicity of the fatty acids to concentration. It has been shown by King and by Frumkin (36) that solutions of the acids from decylic upwards do not come into equilibrium with their surfaces until some considerable time has elapsed, decylic acid requiring from 15 to 30 minutes and dodecylic acid several hours, and it is noteworthy that it is at this part of the series that toxicity begins to decline. A few preliminary experiments on the surface tension of some of these higher acids from decylic upwards, using a Traube stalagmometer, showed that under conditions in which only a short time is allowed for equilibrium to be established, the surface tensions of saturated solutions of decylic and undecylic acids were materially lower than that of pure water, whereas solutions of lauric, palmitic and oleic acids were scarcely different. The decline in toxicities of these acids could not be correlated with the surface tensions of their solutions, for whereas lauric and oleic acids have insecticidal values of a fairly high order, palmitic acid is not materially toxic at high concentrations. Before any correlation between capillary activity and toxicity can be justifiably sought, it will be necessary to ascertain the relative effect upon the surface tension of water of the fatty acids under conditions more closely similar to those prevailing in our insecticide trials, where, in general, emulsions in 1 per cent. saponin solutions were used. The apparent decline in capillary effect of the solutions of the higher acids merely indicates that the molecules of these acids are so sparsely distributed in the solution that a considerable time is required for them to concentrate in the surface. But seeing that toxicity begins to decline at this point, the question already raised as to whether the toxicity of the fatty acids is not in some way

partly bound up with water-solubility relationships, is again brought into prominence.

#### GENERAL CONSIDERATIONS.

There is little probability of finding one simple generalisation capable of explaining the variations in toxicity of the many compounds examined. The particular physiological actions to which compounds give rise are dependent upon chemical constitution. The type of the molecule will therefore have a paramount influence upon toxicity, but physical properties will play an important part in modifying and regulating the intensity of toxic action. But as many physical properties are colligative with molecular constitution, we are presented for each group of chemical compounds with a complex of physical phenomena whose bearing on toxicity is difficult to analyse. Cross-relationships between different groups of compounds are still more difficult to detect, and our present knowledge is not sufficient to enable us to relate toxicity to insects with any one type of molecular structure or any one physical property. Thus, although it is possible that the gradations in toxicity found among the fatty acids may be referred to change in some one physical property as the series is ascended, it is unlikely that such an explanation will be valid for the differences in insecticidal action found between tetramethyl- and tetraethylammonium, or between the nitrophenols, or the aromatic amines. For example, if the relative lowering of the surface tension of their aqueous solutions determines the readiness with which any fatty acid penetrates and therefore its degree of toxicity, this property would not seem to determine the toxicity of dinitrophenol or diphenylamine, the surface tensions of whose saturated solutions as determined by Traube's stalagmometer, are little different from that of pure water (71.8 and 71.2 dynes/cm. at 25° C. respectively as against water 71.8 dynes/cm.).

Solubility relationships both with organic and aqueous media may be one of the factors deciding the degree of toxicity shown by organic compounds. It is perhaps suggestive that in addition to the hydrocarbon ring or chain, most of the compounds exhibiting toxic properties to any marked extent have groups polar to water, a few of them are soluble in water, *e.g.* nicotine and tetramethylammonium chloride. Such polar groups are the radicals OH, COOH, NH<sub>2</sub>, NH. Nevertheless, it would appear impossible in such a way to explain the differences in toxicity between tetramethyl- and tetraethylammonium and between 2 : 4-dinitrophenol and *o*-nitrophenol.



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Shafer<sup>(37)</sup> has shown that many of the more commonly used insecticides kill by their toxic action on the enzymes, but no data exist indicating if this be true for compounds of widely different chemical structure. The data presented here point to the necessity of simplifying the problem of the relationships of chemical constitution and insecticidal action by a study in the first instance of (a) the toxic effects our chemical compounds have on the oxidases, reducases and catalases found in insects, and (b) the differences shown by them in the rate of penetration of chitinous and other membranes. A correlation between physical properties and both phenomena might then be sought.

### SUMMARY.

1. The various classes of insecticides are outlined, and the sense in which the term "contact insecticide" is used is defined as one which is brought into external contact with the insect, either as solid, liquid or vapour.

2. An analysis is made of the relationships between chemical constitution and insecticidal action in the vapour phase. There is rough correlation between both the molecular weights and volatilities of organic compounds and toxicity, but it is probable that these relationships are only indirectly involved and that they indicate a connection of a more direct kind with some other property such as adsorption.

3. An account is given of the toxicity to insects of certain plant products. The most potent of these are certain tropical leguminous plants used as fish-poisons. A brief account is given of the chemical derivatives found in these plants. One of them, "tubatoxin," is one of the most potent contact insecticides known.

4. A list of the groups of organic chemicals tested for their toxic action on *Aphis rumicis* and the eggs of *Selenia tetralunaria* is given. A more detailed account is given for each group of the relationships between chemical constitution and insecticidal action. It is shown that the substitution of certain radicals in the benzene ring profoundly affects toxicity, but that toxic action depends not only upon the radicals but the number substituted and in certain cases upon their relative position.

5. 3 : 5-Dinitro-*o*-cresol is shown to have a most powerful ovicidal effect.

6. An examination of the toxicity of the fatty acids is made. It is shown that as the series is ascended toxicity increases up to undecylic acid, after which it declines.

An analysis is made of the bearing of certain of the physical properties of these acids upon toxicity; such are volatility, physical state, partition coefficients, dissociation constants and surface tensions of their solutions in water. None of these properties entirely accounts for the toxicities shown by the fatty acids, but to a certain extent with some of them correlation is sufficiently close to indicate the necessity of further study but on simplified lines.

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# NUTRITIVE VALUE OF PASTURE.

## II. SEASONAL VARIATIONS IN THE PRODUCTIVITY, BOTANICAL AND CHEMICAL COMPOSITION, AND NUTRITIVE VALUE OF PASTURAGE ON A HEAVY CLAY SOIL.

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(With Two Text-figures.)

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### INTRODUCTION.

THE objects of this series of investigations into the problems connected with the nutrition of animals on pasture have been explained fully in an initial communication(1), which dealt with the nutritive properties of medium pasturage on a light sandy soil under conditions resembling those of close and heavy grazing. It will not be feasible, within the limits of a short introductory section, to summarise in their entirety the numerous conclusions which were drawn from the 1925 season's investigation, and the writers will therefore content themselves at this stage with drawing attention to certain of the more significant findings. Reference to other findings will necessarily be made when discussing the relation of the results of the present experiment to those of the 1925 investigation.

It was demonstrated in the first communication of this series that pasture grass kept short by efficient grazing contains a very high percentage of protein *throughout the whole season*, the amount being more than twice as great as that contained in grass from the same field which had been permitted to grow unchecked to the stage of maturity suitable for hay. On the other hand, the amount of fibre in such young pasture grass is appreciably smaller than that present in meadow hay. In respect of digestibility, well-grazed pasture herbage compares favourably with a concentrate like linseed cake and is far superior to the very best quality of meadow hay. Even the fibrous constituent, which in hay is frequently woody and of low digestibility, is, in pasture grass, digested to an extent almost equal to that of the carbohydrate fraction. The conclusion was reached that young pasture grass possesses a much higher nutritive value than has hitherto been suspected. When properly grazed, its dry matter possesses the character of a protein concentrate of high digestibility and feeding value, and not of a coarse fodder like meadow hay. Unlike many farm concentrates, it is capable of supplying the animal's requirements for vitamins and, where the botanical character of the herbage is satisfactory, as in mixed grass and clover herbage, for bone-forming minerals like lime and phosphate also. It was not without reason, therefore, that the conclusion was drawn that "the farmer's cheapest, and possibly his best, concentrate is to be found growing within reach of the homestead." Young pasture grass is essentially a feeding stuff for production, rather than for maintenance purposes.

It was further shown that, contrary to the view usually held, the high nutritive value of pasturage can, under an efficient system of management, be maintained throughout the season. The grass cut in September and October was little inferior in respect of composition and feeding value to that available in spring. Because of the narrow nutritive ratio, comparable with that of linseed cake, which characterised the pasture ration, it was emphasised that the supplementing of pasturage with linseed cake or other protein concentrates was not in accordance with the scientific principles of feeding, and that for this purpose carbohydrate-rich foods like cereals should be employed. This view was further substantiated by a consideration of the seasonal changes in the lime and phosphate content of the pasture grass, since during the dry mid-season, when supplementary feeding is likely to be necessary, it was found that the grass contained a big excess of lime over phosphate. It is possible to rectify such ill-balance in the mineral content by the

supplementary feeding of cereals and roots, which contain an excess of phosphate over lime.

The object of the present investigation was to ascertain whether the conclusions in regard to the composition and digestibility of pasture herbage on a sandy soil held good for pasturage of different botanical composition on a soil of essentially different characteristics, namely, a heavy clay soil. It was felt that such repetition was desirable before the somewhat unexpected results of the 1925 enquiry could be accepted with entire confidence. For that reason, the 1926 experiment may be regarded as a repetition of the earlier work under different conditions in respect of soil, weather and botanical character of pasturage. Attention was paid as before to securing information on the following specific points:

- (1) The seasonal productivity of the pasture and its correlation with meteorological conditions.

- (2) The changes in the botanical composition of the pasture during the season of experiment.

- (3) Comparison of the productivity of the pasture plot with that of contiguous hay plots.

- (4) The seasonal variations in chemical composition of the pasture cuts. Comparison of these data with figures for hay and aftermath cuts from the meadow plots.

- (5) The seasonal variations in the digestibility and nutritive value of the pasture cuts. Supplementary feeding of grazing animals.

- (6) The seasonal variations in the lime and phosphate content of the pasture herbage.

- (7) The utilisation of the lime and phosphate constituents by sheep.

- (8) The maintenance requirements of adult sheep.

#### GENERAL ARRANGEMENT OF INVESTIGATION.

The present investigation was carried out on a pasture situated on the lower and heavier soil of the University Farm, Cambridge. Immediately underlying the soil of the pasture is a layer of gault clay. The field has the reputation of providing the best heavy land pasturage on the farm. It produces a good crop of grass in most seasons and is characterised by displaying a steady growth through the summer months. It is grazed exclusively by the dairy herd.

The field has been down to grass for a number of decades, the old ridges and furrows still being plainly visible. No artificial fertilisers have been applied during the last four years. It has, however, been enriched

continuously by the droppings of well-fed milking cows, which graze the herbage every year and often receive green crops which are fed to them on the field.

An oblong plot comprising an area of 1.4 acres was marked out on the field for the purposes of the investigation. This area was entirely clear of hedges and trees and was so disposed that the furrows ran parallel with the longer sides. It was further divided up into seven equal sub-plots, each measuring therefore 0.2 of an acre.

Preparatory to the experiment, the selected plot was harrowed on two occasions during February 1926, and some of the roughage left from the previous autumn was removed by means of the grass cutter. The plot was next rolled on March 4 and the motor mower was taken over the whole plot with the knives set high. This resulted in the removal of herbage to the amount of about 96 lb. of dry matter per acre. Between March 10 and 20, the plot was cut over in both directions and almost the whole of the winter grass was removed in these operations, the weight of mown herbage amounting to about 389 lb. of dry matter per acre. Regular daily cutting was begun on March 22, and although the samples still contained a small proportion of old grass, by far the greater bulk consisted, even in the first week, of newly grown herbage. As in the 1925 investigation, cutting was done by a 16 in. "Atco" motor mower fitted with a specially designed grass box. The precautions which were taken in the cutting of the sub-plots, and the question as to how far such a system of cutting may be regarded as imitating actual grazing conditions, have been discussed in detail in the first publication of this series and need not therefore be dealt with at this point. One sub-plot was cut per day and thus the whole plot was cut over once per week. By the action of the motor mower, the herbage was mown as short as it might be left after being eaten down by sheep under conditions of heavy stocking.

The grass resulting from the cutting of a sub-plot was weighed and sampled immediately for analytical purposes. A suitable bulk was then transported to the metabolism building for feeding to two wether sheep, with which digestion and balance trials were carried out. In order to

Table I. *Division of grazing season into periods for purpose of digestion trials.*

Period	1	2	3	4	5	6	7	8	9	10
Date (1926)	Apr. 9	Apr. 27	May 14	June 1	June 18	July 9	July 30	Aug. 20	Sept. 10	Oct. 1
	to	to	to	to	to	to	to	to	to	to
	Apr. 26	May 13	May 31	June 17	July 8	July 29	Aug. 19	Sept. 9	Sept. 30	Oct. 21

obtain a continuous record of the seasonal alterations in the composition and nutritive value of the herbage, the season was divided into ten periods, each period corresponding with the duration of a digestion trial. The dates of these trials are given in Table I.

#### EXAMINATION OF SOIL CONDITIONS.

Any attempt to compare productivity on different types of grassland is necessarily incomplete if it does not take soil conditions into account. Accordingly, after the completion of the trial in late October, the soil was sampled in a number of places to a depth of 9 in. by the usual means, due regard being paid to ensuring that the bulk sample was, as far as possible, representative of the whole plot. The analytical results are tabulated in Table II.

Table II. *Analysis of soil sample from pasture plot.*

Mechanical analysis	%	Chemical analysis (moisture content = 4.75 %)	%
Stones in original sample ...	2.74	Total nitrogen ...	0.497
Soil, after removal of stones:		Nitrate nitrogen ...	0.0035
Moisture ...	4.75	Total $P_2O_5$ ...	0.188
Organic matter ...	15.10	Do. sol. in 1 % citric acid ...	0.062
Fine gravel, 3-1 mm. ...	1.45	Total $K_2O$ ...	0.548
Sand, 1-0.2 mm. ...	12.94	Do. sol. in 1 % citric acid ...	0.004
0.2-0.04 mm. ...	10.71	CaO ...	2.16
Silt, 0.04-0.01 mm. ...	13.39		
0.01-0.002 mm. ...	15.79		
Clay, under 0.002 mm. ...	21.17		

The figures in the first column of Table II disclose a set of soil conditions strikingly different from that which characterised the soil of the 1925 pasture. On the gault clay pasture of the present investigation, the soil contained about 40 per cent. of clay and fine silt and about 23 per cent. of sand, whereas the soil of the sandy pasture of 1925 contained as much as 60 per cent. of sand and only about 11 per cent. of clay and fine silt. Moreover, the 1926 soil was appreciably richer in organic matter than that of 1925 and also contained, as would be expected, a higher percentage of hygroscopic moisture. It was justifiable to conclude that, unlike the 1925 pasture, the present field would not suffer seriously during short spells of droughty weather and would bear out its reputation of being capable of displaying a steady growth through the summer months.

A study of the data obtained in the chemical investigation of the soil shows that it contained rather more nitrogen and nitrate, as well as somewhat bigger percentages of total and available phosphate, than the



1925 soil. The percentage of total potash was distinctly higher, though at the same time this constituent was less available, than in the previous year's sample. It should further be noted that the clay soil contained much bigger reserves of chalk than were found in the sandy soil.

#### WEATHER CONDITIONS DURING THE SEASON OF EXPERIMENT.

Since one of the objects of this series of investigations is to gain an insight into the influence of meteorological conditions on the seasonal productivity of pastures, due attention was paid to keeping an account of the weather data during the period of experiment. In Table III is given a summary of the general meteorological conditions during the grazing season of 1926. More detailed figures will be found in the section dealing with the seasonal productivity of the pasture. The readings were taken at the Meteorological Section of the University Farm within a short distance of the experimental pasture. In view of the somewhat lower situation of the pasture in relation to the station, it is probable that the temperature data given in Table III may be rather higher than would have been obtained if the readings had been taken on the plot itself.

Table III. *General meteorological conditions during the grazing season of 1926.*

Month ... ..	April	May	June	July	Aug.	Sept.	Oct.
Rainfall in inches ...	2.86	1.93	2.56	2.18	1.22	2.47	2.33
Average per day ...	0.095	0.062	0.085	0.070	0.040	0.082	0.075
No. of rainy days (0.01 in. or over)	18	20	16	13	12	10	14
Sunshine in hours ...	108	164	182	166	193	146	114
Average per day ...	3.6	5.3	6.1	5.4	6.2	4.9	3.7
No. of sunless days (1 hr. or less)	8	4	5	3	4	4	7
No. of absolutely sunless days	4	0	3	2	1	3	5
Mean max. daily temp. ° F.	57	59	65	71	72	68	54
Mean min. night temp. ° F.	41	43	49	54	53	52	40

The data given in Table III show that the weather conditions prevailing during the 1926 experiment were in marked contrast to those which characterised the grazing season of 1925. Reference to the corresponding table in the previous publication reveals the fact that the mid-season of 1925 was an extremely sunny and droughty period, and this circumstance exercised a very pronounced influence on the seasonal productivity of the light-land pasture.

Rainfall during the 1926 season was not only more copious, but was also better distributed over the season from the point of view of growth.

It was not until autumn that any prolonged spells of dry weather occurred, August, and not June as in 1925, being the driest and sunniest month of the season. In September there were as many as twenty days on which no rainfall was registered, the period from September 8 to 23 (inclusive) being absolutely rainless. A large proportion of the September rain fell on a single day, namely September 2, when over one inch of rainfall was recorded. Sixteen days in October were rainless, though during the first half of this month a series of heavy dews largely compensated for such conditions.

The 1925 and 1926 seasons further differed markedly in regard to temperature records. April and May in 1926 were abnormally cold, the maximum day temperatures right up to the end of May ranging between 50 and 60° F. June was by no means a normally warm month. In the second half of October, a series of night frosts brought growth on the pasture almost to a standstill and resulted in the experiment being brought to a conclusion at an earlier date than was the case in the 1925 trial.

The distinctive weather conditions in the 1926 grazing season may thus be summarised: an exceedingly cold and wet spring; adequate rainfall during mid-season; a fairly dry autumn; early frosts in October.

#### BOTANICAL NOTES.

Careful surveys of the pasture plot were made from time to time throughout the season of the experiment with a view to establishing the trend of the botanical changes and ascertaining the nature of the ration which was being fed to the sheep in the digestion trials. For this purpose, the writers were fortunate enough to secure the services of Mr S. F. Armstrong, M.A., who kindly furnished the reports which are summarised in Table IV. The percentages given in this table represent, in an approximate sense, the proportions of the different species which were included in the samples cut by the mowing machine. It was assumed that with such closely cut pasture the percentages by weight of the different species present in the sample after cutting would be very similar to the percentage areas occupied by the same species. With longer herbage, this assumption would naturally not hold.

In addition to these occasional detailed surveys, notes were made from day to day of any salient features which might be of significance when seeking to interpret the results of the digestion trials.

Table IV. Showing botanical character of pasture herbage at different stages of grazing season (grasses given in order of frequency of occurrence).

March 10, 1926	May 28, 1926	July 3, 1926	September 30, 1926	October 25, 1926
<ol style="list-style-type: none"> <li>1. <i>Poa trivialis</i> (rough-stalked meadow grass)</li> <li>2. <i>Lolium perenne</i> (perennial rye grass)</li> <li>1. and 2. formed about 70 % of herbage, with 1. markedly predominant</li> <li>3. <i>Agrostis alba</i> (creeping bent) } roughly 15 %</li> <li><i>Cynosurus cristatus</i> (cocktail) } 15 %</li> <li><i>Dactylis glomerata</i> (dogtail) } 15 %</li> <li><i>Pheum pratense</i> (Timothy) } 15 %</li> <li><i>Alopecurus pratensis</i> (foxtail) } traces</li> <li><i>Festuca rubra</i> (red fescue) } traces</li> <li>Wild white clover: only small amount</li> <li>Wild white clover: only small amount</li> <li>Weeds: bulbous buttercup, daisy, plantain, dandelion (comprising about 15 %)</li> </ol>	<ol style="list-style-type: none"> <li>1. <i>Poa trivialis</i> (roughly 50 %)</li> <li>2. <i>Agrostis alba</i> v. <i>stol.</i> }</li> <li><i>Cynosurus cristatus</i> }</li> <li><i>Lolium perenne</i> }</li> <li><i>Dactylis glomerata</i> }</li> <li><i>Pheum pratense</i> }</li> <li><i>Alopecurus pratensis</i> }</li> <li><i>Poa annua</i> (annual meadow grass) }</li> <li><i>Festuca pratensis</i> (meadow fescue) }</li> <li><i>Holcus lanatus</i> (Yorkshire fog) }</li> <li><i>Festuca rubra</i> }</li> <li><i>Avena flavescens</i> (golden oat grass) }</li> <li>Weeds: roughly 15 %</li> <li>Wild white clover: increasing slightly</li> </ol>	<ol style="list-style-type: none"> <li>1. <i>Agrostis alba</i> v. <i>stol.</i> (creeping bent)</li> <li>2. <i>Poa trivialis</i></li> <li><i>Lolium perenne</i> }</li> <li><i>Cynosurus cristatus</i> }</li> <li><i>Dactylis glomerata</i> }</li> <li><i>Alopecurus pratensis</i> }</li> <li><i>Pheum pratense</i> }</li> <li><i>Holcus lanatus</i> }</li> <li><i>Hordeum pratense</i> }</li> <li><i>Poa annua</i> }</li> <li>Weeds: diminishing in amount</li> <li>Wild white clover: increasing on tops of ridges</li> </ol>	<ol style="list-style-type: none"> <li>1. <i>Agrostis alba</i> v. <i>stol.</i> (roughly 65 %)</li> <li>2. <i>Poa trivialis</i> (roughly 5 %)</li> <li><i>Lolium perenne</i> }</li> <li><i>Cynosurus cristatus</i> }</li> <li><i>Dactylis glomerata</i> }</li> <li><i>Holcus lanatus</i> }</li> <li><i>Pheum pratense</i> }</li> <li><i>Alopecurus pratensis</i> }</li> <li>Red clover</li> <li>Wild white clover: roughly 8 %</li> <li>Weeds: roughly 7 %</li> </ol>	<ol style="list-style-type: none"> <li>1. <i>Agrostis alba</i> v. <i>stol.</i> (roughly 90 %)</li> <li>2. <i>Poa trivialis</i> beginning to revive</li> <li>Traces of other grasses as before</li> <li>Wild white clover: very small leaves and not so plentiful as on September 30</li> <li>Weeds: somewhat scarcer and less virile, with exception of daisies</li> </ol>

*Comments on Table IV.*

*Agrostis alba* v. *stol.* This species was easily recognisable among the herbage growing in the pasture at the commencement of the experiment in March. Together with dogtail and cocksfoot, it constituted about 15 per cent. of the herbage cut by the mower. By the beginning of July, creeping bent had so increased in amount as to be the dominant grass in the pasture. During the subsequent part of the season, this species underwent tremendous development, and it was estimated that the herbage during October contained as much as 90 per cent. of creeping bent. Concurrently with this enormous increase of bent grass was noted a continuous falling off in the amount of rough-stalked meadow grass and perennial rye grass, the productivity of these grasses being affected adversely by the competition of the stronger growing *Agrostis*. It should be borne in mind that the conditions of the experiment were very favourable to the development of *Agrostis*, this grass being scarcely cut at all in its early stages on account of its creeping habit. Such ideal conditions would not have obtained had this grass been pulled up and bitten off as it would be by a grazing animal.

*Poa trivialis.* At the beginning of the season, this species was easily the most conspicuous grass in the pasture plot. It continued to grow freely during the early part of the trial, so that at the end of May it still formed about 50 per cent. of the ration fed to the sheep. By the end of June, however, it was beginning to feel the severe competition of *Agrostis*, the latter having now taken precedence as the main grass in the pasture. From this time on, *Poa trivialis* diminished in importance, and at the end of September did not constitute more than 5 per cent. of the herbage. At this stage, as a result of a fairly dry autumn and the competition of *Agrostis*, the distribution of the shallow rooting *Poa trivialis* was indicated by numerous small brown patches in the plot. These dry patches revived somewhat under the influence of the rains of October. It was noteworthy that even on this heavy clay pasture, which on accepted theories should not suffer from lack of water-retaining power, comparatively short spells of dry sunny weather always resulted in the perceptible "scorching" of *Poa trivialis*. This effect may not wholly have been due, however, to the effect of moisture scarcity in the topmost layer of soil. The natural habitat of *Poa trivialis* is in semi-shade, e.g. under or near trees and hedges. It is undoubtedly a greater success as a meadow than as a grazing plant. The steady decline of this species as the season advanced may therefore have been due in some

measure to the effect of close cutting, which led to the absence of shading effect from contiguous herbage and left the plant fully exposed to light, a condition which is usually unfavourable to the free growth of this species.

*Lolium perenne*. One of the most striking features of the botanical surveys was the steady diminution in the amount of perennial rye grass in the pasture plot as the season progressed. This species figured fairly prominently during March; by the beginning of September, however, it had sunk to quite an insignificant position and, in combination with crested dogtail and cocksfoot, helped to form only about 10 per cent. of the ration.

*Dactylis glomerata*. This species held its position throughout the season as one of the secondary grasses in the plot. In the meadow plots, it was the first of the grasses to display recovery after mowing for hay, and throughout the rest of the season was much more prominent and vigorous in the meadow plots than in the pasture plot. This observation is in harmony with Stapledon's conclusion that heavy grazing in early spring reacts adversely on the later productivity of bulky grasses like cocksfoot (2).

*Wild white clover*. The amount of clover in the pasture at the beginning of the experiment was insignificant. Small patches could be noted at various points, such patches being more in evidence at the northern end of the plot than at the southern. By the end of May, the clover plants were beginning to display vigorous growth and the patches were spreading. During June and July, white clover flourished and spread very appreciably, so that during early August it formed about 10 per cent. of the herbage cut by the mower. This stage appeared to mark the maximum phase of clover development, however, and from this point onwards it dwindled rather than increased in amount. Towards the end of the experiment in October, it did not form, partly on account of its very small leaves at this stage, more than about 5 per cent. of the grass ration.

The frequent and close cutting of the plot undoubtedly exerted a beneficial influence on the development of wild white clover. This was clearly brought out by a comparison of the pasture plot with the meadow plots; in the latter, clover plants were very scarce. In many parts, clover leaves could be discerned plentifully right up to the edge of the pasture plot and, in a sharply defined manner, these became absent or scarce as the observer passed over the boundary of the plot into the unchecked growth on the rest of the field. Here the shading effect of

the longer grasses appeared sufficient to check the free development of the dwarf clover plants.

It should be emphasised, however, that the growth of clover on this heavy clay pasture was in no sense so luxuriant as was noted on the light sandy pasture in the 1925 experiment, despite the circumstances that (1) the clay soil contained bigger reserves of chalk and available phosphate than did the sandy soil, and (2), other things being equal, strong clay soils are, according to general experience, the most favourable to growth of wild white clover.

The failure of white clover to increase on the same marked scale as was noted in the 1925 pasture may be due to the following reasons: (1) clover plants in the present pasture were, at the beginning of the experiment, only present in certain small well-defined areas. They were by no means generally distributed throughout the plot. (2) The dominant grass in the 1926 pasture was *Agrostis alba* v. *stol.*, a species with a creeping habit of growth not unlike that of wild white clover. It is probable that the free development of clover was checked by the abundant and vigorous growth of *Agrostis* and that the gradual diminution in the amount of clover after a period of maximum luxuriance in early August was the consequence of the particularly severe competition of *Agrostis* during the second half of the season.

In his early botanical surveys of old and recent pastures in the English Midlands, Armstrong<sup>(3)</sup> noted that very little white clover was to be found in fields where *Agrostis* constituted the main form of herbage. It appears reasonable to conclude that wild white clover and *Agrostis alba*, possibly on account of their similar habits of spreading, do not flourish well together in a pasture, and that the abundant growth of

Table V. *Showing botanical characteristics of 1925 and 1926 pasture plots.*

Species	1925 pasture (light sandy soil)	1926 pasture (heavy clay soil)
<i>Lolium perenne</i>	Conspicuous during whole season	Of minor importance
Wild white clover	Abundant growth	Moderate growth
<i>Poa trivialis</i>	Abundant, especially during later stages of season	Abundant up to end of May; of minor importance during second half of season
<i>Agrostis alba</i> v. <i>stol.</i>	Abundant during autumn	Dominant species
<i>Bromus mollis</i>	Abundant during spring; absent during autumn	Absent

the latter species reacts adversely on the development of the former. On the other hand, however, it is possible that the converse may hold also, namely, that the stimulation of clover may tend to check the growth of *Agrostis*, an idea which may possibly explain attempts which have

been made in the past to reduce the amount of *Agrostis* in pastures by broadcasting seed of wild white clover. It was certainly noted that clover was most plentiful in those parts of the 1926 pasture plot where *Agrostis* was least abundant.

It is of interest at this stage to compare the main botanical characteristics of the 1926 pasture with those of the 1925 plot. The comparison is shown in Table V.

The observations summarised in Table V become significant when considered in relation to Armstrong's findings in regard to the botanical composition of the herbage on different types of pasture in the English Midlands(3). Armstrong concluded that white clover and rye grass formed by far the greater part of the herbage on the best grazing lands, and that the next most abundant species on these pastures were usually *Cynosurus cristatus*, *Agrostis stolonifera* and *Poa trivialis*. The herbage on the inferior grasslands consisted largely of *Agrostis vulgaris* and various weeds, whilst white clover and rye grass were present in comparatively small quantities.

It will be noted that the botanical characteristics of the 1925 pasture were such as might be possessed by grazing land of superior properties. The results of the 1925 investigation certainly demonstrated the excellent nutritive properties of the herbage under a system of close cropping. On the other hand, however, rye grass and white clover were present in relatively small amount in the 1926 pasture, whilst *Agrostis alba* v. *stol.* was specially abundant. Though the presence of small amounts of this last-named species in pastures is not inconsistent with good nutritive properties, and indeed is probably to be desired on account of the capacity of *Agrostis* for yielding luxuriantly late in the season, yet the botanical composition of the 1926 pasture was such as would formerly have been associated with an inferior grassland. With this view Mr Armstrong himself was in agreement.

The results of the 1926 investigation therefore take on a special interest, in that it becomes possible to institute a comparison, under the same system of management, between the nutritive properties of two pastures which would, regarded merely in the light of botanical composition, be considered to possess widely differing feeding properties. It should incidentally be possible to discover whether good management in regard to stocking is able to compensate for any disadvantages which may attach to inferior botanical composition.

A summary of the notes made of the salient features of the plot from period to period is given below.

*Period 1* (April 9 to April 26). At the commencement of the experiment, dandelion heads were beginning to appear and the seed heads of the earlier grasses were being formed. White clover was quite negligible in amount. Throughout the period, the tops of the blades of grass had a pinched and brown appearance, due probably to the cold wet weather which prevailed during the preliminary cutting of the plot prior to the start of the experiment proper. Only in the hollows, where a certain amount of shelter was possible, did growth of grass proceed at all satisfactorily.

*Period 2* (April 27 to May 13). The early days of this period marked a vigorous development of the weeds, particularly of dandelions and buttercups, which sprang up rapidly again after being cut down by the machine. Isolated patches of clover were beginning to spread somewhat, and *Poa annua*, which had established itself in any small bare patches on the plot, was now flowering. Several large "fairy rings" were producing luxuriant herbage of dark colour which grew at approximately twice the rate of grass not so affected. This growth was so plentiful, that it was necessary right up to August to take the machine over such herbage twice in order to keep it at the same level as the other grass in the sward.

*Period 3* (May 14 to May 31). At the beginning of this period, the grass was still of poor colour and the yield had fallen somewhat. Later, however, the colour of the herbage improved and white clover, especially at the northern end of the plot, began to display vigorous growth, though at this stage the mower was not cutting much of this. Buttercups and daisies figured prominently in the mown herbage.

*Period 4* (June 1 to June 17). White clover spread appreciably in all the sub-plots during this period, the plants being large enough to be cut and included in the samples for feeding to the sheep. Weeds were not so plentiful; in particular, buttercups were absent in all save the damper parts of the plot.

*Period 5* (June 18 to July 8). Flower stalks of *Hordeum pratense* were appearing in numbers in the furrows and persisting. Clover was still present in fair amount. At the beginning of July, the "fairy rings," which up to now had been very luxuriant, were beginning to reveal bare brown patches among the dark green grass of the ring.

*Period 6* (July 9 to July 29). White clover underwent further increase and the herbage of the plot now appeared succulent and of good colour. Towards the end of the period it was noted that the bottom grass, consisting mainly of *Poa trivialis*, was becoming "burnt." *Dactylis*



*glomerata* was not affected at all and for that reason probably constituted a larger proportion of the mown herbage than at any other time.

*Period 7* (July 30 to August 19). The "burning" effect on the *Poa trivialis* was still noticeable at the southern end of the plot.

*Period 8* (August 20 to September 9). This was the driest period so far, and the average moisture content of the herbage would have been still lower but for two or three very wet days towards the close of the period. Leaves were falling on to certain of the sub-plots from a neighbouring elm tree and this necessitated the employment of boys to pick them up prior to cutting operations. No elm leaves were contained in the rations as fed to the sheep. Trouble was also beginning to be experienced with worm casts.

*Period 9* (September 10 to September 30). During this period, worm casts were included in the mown samples to a significant extent. *Poa trivialis* was not flourishing and showed up as numerous small brown patches. This was scarcely surprising in view of the fact that from September 8 to September 23 (inclusive) no rainfall at all was registered.

*Period 10* (October 1 to October 24). The yield, which had fallen off somewhat during Period 9, improved again during the early part of this period, probably as a result of a succession of very heavy dews.

*Poa trivialis* revived a little, but *Agrostis alba* formed almost the whole of the mown herbage. White clover was too small in the leaf to be cut in large quantity. Worm casts were plentifully included in the samples, together with a proportion of dead grass stems. During the final week, a succession of heavy frosts brought growth almost to a standstill.

#### SECTION I. SEASONAL VARIATIONS IN THE PRODUCTIVITY AND CHEMICAL COMPOSITION OF THE PASTURE.

A summary of the yields of grass from the pasture plot is given in Table VI. The data are expressed in terms of lb. of dry matter per acre per week and refer to soil-free grass. Together with these data are shown, also in terms of dry matter per acre of soil-free grass, the yields from certain contiguous plots from which a hay cut and a number of weekly aftermath cuts were taken.

In order to ascertain whether the pasture plot was reasonably uniform in respect of productivity, the yields of dry matter from the individual sub-plots are summarised in Table VII.

It is interesting to note that the difference in the yields of the best and worst sub-plots was about 6 per cent., a degree of variation equal

to that noted among the sub-plots of the light-land pasture of 1925. In the present instance, the productive capacities of sub-plots 1 to 5 were strikingly uniform; at the northern end of the pasture plot, where

Table VI. *Yields in lb. dry matter per acre of pasture and hay plots.*

Week ending	Pasture plot	Hay plots	Week ending	Pasture plot	Hay plots	Week ending	Pasture plot	Hay plots
March 28	89.4	—	June 13	146.3	—	Aug. 22	113.7	77.6
April 4	76.0	—	20	130.1	—	29	96.6	78.6
11	126.0	—	27	130.2	5190	Sept. 5	128.8	108.4
18	103.3	—	July 4	129.5	—	12	182.9	141.0
25	94.2	—	11	170.4	1293	19	119.8	97.1
May 2	126.0	—	18	191.9	106.0	26	80.1	68.2
9	116.8	—	25	142.0	75.0	Oct. 3	79.4	56.2
16	94.3	—	Aug. 1	144.0	80.0	10	63.6	50.0
23	91.6	—	8	123.9	65.2	17	82.6	86.1
30	147.2	—	15	127.7	65.2	24	75.5	52.6
June 6	143.4	—						

	Pasture plot lb. per acre	Hay plots lb. per acre
Total dry matter produced during whole trial ...	3667.2	7690.2
Mean dry matter content of grass as cut from pasture plot = 21.96 %.		

Table VII. *Yields during whole season from pasture sub-plots.*

Sub-plot	Yield in lb. dry matter per $\frac{1}{2}$ acre
1	734.0
2	738.0
3	742.0
4	755.0
5	735.0
6	717.0
7	712.0

the percentage botanical composition was slightly different from that of the rest of the sward, the amount of herbage grown during the season was somewhat lighter.

#### *Comments on Table VI.*

*Seasonal productivity of the pasture plot and the influencing factors.*  
It will be recalled that very definite correlations were found to exist between seasonal productivity of the pasture and the prevailing meteorological conditions during the 1925 period of experiment. The observed facts are briefly restated below:

1. During the course of the droughty season lasting from the beginning of June till the end of August, there was a distinct correlation between rainfall and yield, the productivity of the pasture increasing or declining in the week following a rise or fall in the amount of rainfall.

This effect was very distinctly displayed and pointed clearly to the controlling influence of rainfall on the productivity of pastures on light soils of low water-retaining capacity during a dry summer.

2. During the early part of the season, a correlation appeared to exist between temperature changes and variations of yield from the beginning of active growth until the date when lack of moisture began to be of supreme importance, a higher mean weekly temperature being reflected in an increased productivity during the same week.

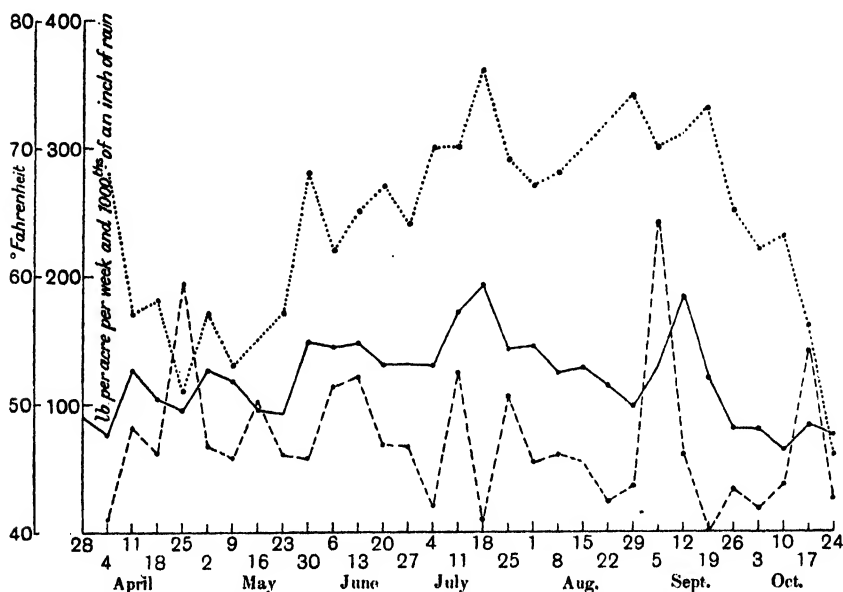


Chart I. Showing records of weekly yields and meteorological records.

— Yield of grass dry matter per acre per week.  
 - - - Mean daily rainfall during a week.  
 ..... Mean weekly day temperature.

3. During autumn, the influence of rainfall on productivity was scarcely perceptible, and the yield of the pasture was much more definitely affected by changes in the day and night temperatures. At the extreme end of the grazing period, the limiting factor appeared to be neither temperature nor rainfall, but rather the diminished hours of daylight.

4. The curve of productivity was characterised by displaying a very pronounced maximum during the month of May. During a period of active growth from May 11 to May 25, nearly 22 per cent. of the season's yield from the pasture was produced. A steady decline of activity

followed, until in mid-July, following a droughty spell of weather, productivity was at its lowest level. A subsequent renewal of activity led to a continuous improvement in the rate of production of herbage from July 20 to August 10. From this date until about October 12, the productive capacity remained fairly steady, although the weekly yield was only about one-fifth of that cut during the "zenith period." As October advanced, the productivity of the plot fell suddenly to a relatively low level, which was maintained almost without change until the end of the trial in early November.

It has been considered necessary to set out the above conclusions somewhat fully, because results of an essentially different nature have been obtained in the present investigation, as will be revealed from an examination of Chart I. The curve of seasonal productivity for the 1926 pasture differs strikingly from that for 1925 in that it exhibits no spring maximum. Although definite spurts in growth of herbage were noted at one or two points of the season, these were not manifested during the early part of the year, nor were they at all comparable in magnitude with that associated with the spring flush of 1925.

It is of importance to enquire into the causes which may have been responsible for the absence of any pronounced spring "zenith period" during the present season's work, since the grazier is accustomed to looking forward to a flush of new growth on his pastures at this time of the year. The possible factors are considered below:

1. Rainfall was abundant and well distributed during April, May and June. Lack of adequate rainfall cannot therefore have been even a contributory cause.

2. An examination of the weather curves in Chart I shows that this part of the year was not only very wet, but also cold beyond the average. Even in a normal season, it would be anticipated that the present pasture, on account of its lower situation and its heavy clay soil, frequently waterlogged in parts during wet winters, would be slower than the sandy pasture of 1925 in becoming warmed up to the temperature necessary to enable the grasses to display that intensive activity which is potential to them in spring. When, in addition, the spring time is both cold and wet, it is scarcely surprising if the early period passes without any manifestation of intensive productivity on such a field.

Despite the absence of a spring "zenith period," the present pasture was, over the whole season, more productive than the 1925 field, yielding, on the basis of one acre, 3667 lb. of dry matter, compared with 3065 lb. for the 1925 plot. This was due to the steady rate of growth throughout

the season, there being no pronounced mid-season drop in productivity such as was noted in 1925. The following considerations may be cited to account for this behaviour: (1) Rainfall during summer was adequate for growth. (2) The water-retaining capacity of the clay soil enabled the pasture to withstand successfully the effects of short spells of dry weather. (3) There was an absence of the customary spring "zenith" activity, so that the grasses did not suffer from the temporary exhaustion of growth power which follows such intense productivity. (4) The presence in the plot of an abundance of *Agrostis alba*, a species which attains to its full luxuriance in late summer and autumn, served to maintain productivity during the second half of the season at a relatively high level.

*Rainfall, temperature and yield.* A detailed study of the graphs drawn in Chart I enables conclusions to be made in respect of the influence of rainfall and prevailing temperature on the productivity of the pasture.

1. *May 23 to July 25.* A sudden rise in yield was noted during the week ending May 30. This could not be ascribed to the influence of increased rainfall during the same or the preceding week and was obviously associated with the sudden rise from low to high daily temperatures during the same week. The influence of these high temperatures was still felt during the succeeding week, since despite a fall in the average temperature for this week, the productivity of the plot showed only an insignificant decline in the week ending June 6. That rainfall at this part of the year was exercising little or no influence on the level of productivity is illustrated by the fact that the steep ascent of the rainfall curve between May 30 and June 13, as well as the steep descent between June 13 and July 4, were without effect on the rate of growth, the latter, which remained remarkably steady over this period, being apparently controlled by the prevailing temperature conditions. Indeed, following the low rainfall in the week ending July 4, productivity suddenly rose to a higher level in the week ending July 11. This was solely the result of the high temperatures registered during the weeks ending July 4 and 11. In the week ending July 18, both temperature and yield curves rose to the highest peaks displayed throughout the season. The high rainfall during the preceding week also contributed to the manifestation of this "zenith period" of growth. A sharp decline in productivity succeeded this week of intensive growth, the result partly of a fall in temperature during the week ending July 25 and partly of a very low rainfall in the week ending July 18.

2. *July 25 to September 12.* The autumn of 1926 was a season of fairly dry weather, interrupted by occasional days of heavy rain. During this period, the influence of rainfall on seasonal productivity became more clearly traceable, temperature conditions throughout being satisfactory for active growth. The portion of the rainfall curve between July 25 and August 22 runs roughly parallel with the yield curve between August 1 and 29. Over this interval, a rise or decrease of rainfall was followed by a rise or fall of yield in the succeeding week. After a spell of dry weather towards the end of August, heavy rain, amounting to 1.60 in., fell on the first three days of September, more than 2 in. of rainfall being registered during the first week of this month. This was undoubtedly responsible for the sharp rise in yield during the week ending September 12.

3. *September 12 to October 24.* The abrupt diminution in yield during the week ending September 19 appears to be associated with the drop in rainfall during the preceding week, since the prevailing temperature still remained high. The week ending September 19 was absolutely rainless, and this, combined with an appreciable fall of temperature in the week ending September 26, occasioned a further decline of yield. Thereafter the days and nights became rapidly colder and increased rainfall had little or no effect on yield.

4. *April 4 to May 23.* It has already been pointed out that during the early part of the season rate of growth was limited by conditions of low temperature and only influenced by rainfall in so far as the presence of an excess of moisture in a clay soil may render it slower in responding to rises in atmospheric temperature. From April 11 to May 23 the ruling temperatures were throughout too low to permit of much growth, and for that reason it was scarcely to be expected that slight variations of temperature during this period would be reflected in increased or decreased yields from the plot. During the week ending April 4, however, relatively high temperatures were registered, and it was justifiable to anticipate that these favourable temperature conditions would lead to increased growth on the pasture. This expectation was realised. The response, however, did not occur in the same week, but in the week following, despite the fact that by this time the prevailing atmospheric temperature had fallen again to an appreciably lower level. Owing therefore to the slowness with which a wet clay soil is heated up, there is likely, during early spring, to be a distinct lag between the incidence of changes of atmospheric temperature and their observed effects on the productivity of pastures situated on such soils. Quite the contrary

effect was noted in the early part of the 1925 season during the investigation of the seasonal productivity of the sandy pasture. In this case, the response to temperature influences was much more prompt, a rise in the mean weekly temperature being reflected in an increased yield during the same week.

*Comparative yields from pasture and hay plots.* Two plots, adjoining the main pasture plot and comprising together 0.1 of an acre, were allowed to grow unchecked to the stage of maturity suitable for hay-making. These plots were mown on June 25 and yielded at the rate of 5190 lb. dry matter per acre. The remaining stubble was removed on July 8 by means of the motor mower, and from July 16 onwards the plots were cut over weekly. The successive weekly yields from the hay plots in terms of lb. dry matter per acre are recorded in Table VI.

The whole season's bulk of produce per acre from the hay plots was more than twice as great as that obtained from the pasture plot, a result very similar to that obtained in the previous year on the sandy pasture, and confirming the 1925 finding that fields kept under meadow conditions produce more heavily than those grazed by animals. The question of the relative production of starch equivalent and digestible protein from the pasture and hay plots will be discussed in Section II.

Subsequent to the cutting of the hay crop, it will be noted that the hay plots never yielded so heavily as did the pasture plot. From July 12 to the end of the season, although the seasonal variations of productivity on the hay and pasture plots ran roughly parallel, yet the hay plots grew only about two-thirds of the dry matter per acre yielded by the pasture plot. This is in harmony with the 1925 observations in this connection, and confirms the conclusion that the taking of a heavy hay crop, with its development of flower heads and seeds, results in a more pronounced weakening of the grasses and a greater reduction of their productive capacity than does continuous grazing.

#### CHEMICAL COMPOSITION OF PASTURE CUTS.

The results of the analysis of the period composite samples of pasture grass are summarised in Table VIII. They represent the mean composition of the dry matter of the rations fed to the sheep during the several periods of the digestion experiment.

#### *Comments on Table VIII.*

The abrupt rise in the percentage of ash in periods 9 and 10 was the result of the inclusion, during these periods, of appreciable amounts

of soil in the grass samples during cutting operations. In order to bring out clearly the effect of season on the composition of the pasture cuts, it has been necessary to recalculate the figures in Table VIII, applying

Table VIII. *Composition of period composite samples of pasture grass (on basis of dry matter).*

Period	...	...	1	2	3	4	5	6	7	8	9	10
			%	%	%	%	%	%	%	%	%	%
Crude protein	...	...	26.65	23.97	21.14	22.68	23.03	25.14	25.46	24.65	25.91	22.20
Ether extract	...	...	5.71	6.02	5.55	7.01	7.04	7.35	6.40	6.27	6.50	5.31
N-free extractives	...	...	40.86	41.63	45.18	41.13	41.37	38.70	39.15	39.30	35.48	38.42
Crude fibre	...	...	15.23	15.68	17.28	18.95	18.37	18.66	19.41	19.59	19.08	16.04
Ash	...	...	11.55	12.70	10.85	10.23	10.19	10.15	9.58	10.19	13.03	18.03
True protein	...	...	21.62	19.30	17.16	18.39	19.52	20.18	20.41	19.50	20.92	18.16
"Amides"	...	...	5.03	4.67	3.98	4.29	3.51	4.96	5.05	5.15	4.99	4.04
Mean moisture as cut			78.8	80.7	79.1	80.1	78.0	78.8	78.1	77.5	76.8	72.5

a correction for the soil content of the samples. As the included soil was composed of worm-casts, a large sample of the latter was collected and submitted to analysis, the data thus obtained forming the basis of the correction.

Table IX. *Analysis of a sample of worm-casts from pasture plot\* (on basis of dry matter).*

	%
Ash	77.80
Organic matter	22.20
CaO	1.15
P <sub>2</sub> O <sub>5</sub>	0.02
SiO <sub>2</sub>	74.40

\* Determinations carried out by methods employed for corresponding determinations on grass samples.

The data in Table IX reveal the interesting fact that although the soil sample from the plot contained about 0.19 per cent. of P<sub>2</sub>O<sub>5</sub>, the amount of this constituent in the worm-casts was almost negligible. The manner of correcting the data in Table VIII for soil content has been described in the first publication and, in effect, the result was to

Table X. *Soil and silica content of pasture cuts.*

Per 100 gm. dry matter of pasture cut

Period	...	1	2	3	4	5	6	7	8	9	10
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Silica		3.45	4.47	4.13	3.41	3.33	2.25	1.48	2.90	4.76	9.68
Dry soil		2.70	4.10	3.63	2.65	2.54	1.06	—	1.95	4.50	11.24
Soil ash		2.10	3.19	2.82	2.06	1.98	0.82	—	1.52	3.50	8.74
Soil CaO*		0.031	0.047	0.042	0.030	0.029	0.012	—	0.022	0.052	0.129
Soil P <sub>2</sub> O <sub>5</sub> *		—	—	—	—	—	—	—	—	—	0.002
Soil SiO <sub>2</sub>		2.00	3.05	2.70	1.97	1.89	0.79	—	1.45	3.34	8.36

\* These figures are utilised in Section III for correcting mineral analyses of grass.



base the figures on a common silica content of 1.48 per cent., this being the lowest percentage of silica obtained in the analysis of the grass samples (period 7). The essential data employed for the purposes of the correction are appended in Table X.

Table XI. *Composition of soil-free samples of pasture grass*  
(on basis of dry matter).

Period ...	1	2	3	4	5	6	7	8	9	10
Mean date (1926) ...	Apr.	May	May	June	June	July	Aug.	Aug.	Sept.	Oct.
	16	3	21	7	28	19	9	30	20	11
	%	%	%	%	%	%	%	%	%	%
Crude protein ...	27.22	24.76	21.75	23.16	23.49	25.35	25.46	25.03	26.85	24.33
Ether extract ...	5.83	6.22	5.71	7.16	7.18	7.41	6.40	6.37	6.74	5.82
N-free extractives ...	41.74	43.00	46.49	41.99	42.21	39.02	39.15	39.91	36.77	42.10
Crude fibre ...	15.56	16.20	17.78	19.35	18.74	18.81	19.41	19.89	19.77	17.57
Ash ...	9.65	9.82	8.27	8.34	8.38	9.41	9.58	8.80	9.87	10.18
True protein ...	22.08	19.94	17.66	18.78	19.91	20.35	20.41	19.80	21.68	19.90
"Amides" ...	5.14	4.82	4.09	4.38	3.58	5.00	5.05	5.23	5.17	4.43

\* Owing to the method of making the grass composites, three days should be added to these dates to give mean dates of the analytical periods of the digestion trials (see Section II).

Table XII. *Composition of hay and aftermath cuts from hay plots*  
(on basis of soil-free dry matter).

No. of cut ...	1	2	3	4	5	6, 7*, 8, 9	10, 11*, 12, 13	14, 15*, 16, 17
Date (1926) ...	June	July	July	July	July	Aug. 6,	Sept. 3,	Oct. 1, 8,
	25	8	16	23	30	13, 20, 27	10, 17, 24	15, 22
	%	%	%	%	%	%	%	%
Crude protein ...	8.84	8.94	18.26	23.41	26.28	27.19	27.09	24.00
Ether extract ...	3.49	2.63	4.06	4.51	5.15	5.82	5.80	4.48
N-free extractives ...	53.97	55.41	47.23	42.71	39.18	38.46	39.29	41.00
Crude fibre ...	29.50	28.40	24.42	22.85	20.80	19.64	19.04	17.85
Ash ...	4.20	4.62	6.03	6.52	8.59	8.89	8.78	12.67†
True protein ...	7.79	6.98	15.09	19.03	20.70	21.22	20.82	19.87
"Amides" ...	1.05	1.96	3.17	4.38	5.58	5.97	6.27	4.13

\* Composite samples made up from aftermath cuts as indicated.

† Presence of worm-casts in this sample rendered this set of figures less trustworthy than the others.

### *Comments on Tables XI and XII.*

*Composition of pasture cuts.* The figures in Table XI afford a decisive confirmation of the conclusions drawn from the results of the 1925 investigation in respect of the composition of pasture grass kept short by frequent cutting or close grazing. The dry matter of the pasture herbage was *throughout* rich in crude protein, the percentage of this constituent varying between 21.75 per cent. in period 3 and 27.22 per cent. in period 1, the mean percentage for the whole season being 24.74 per cent. The extreme values in the 1925 investigation were 21.20 and 27.92 per cent., again with an average value for the whole season

of 24.74 per cent. This strikingly good agreement between the results of the two investigations in this regard is all the more noteworthy in view of the fact that the two types of herbage were essentially different botanically and that the 1925 pasture displayed a much more luxuriant growth of wild white clover than was noted on the 1926 plot. It is justifiable to conclude that, under a system of close grazing, a pasture will yield, irrespective of botanical composition, a produce whose dry matter will be exceedingly rich in protein, and further that the high content of protein will be maintained throughout the entire season. Only when the herbage is inadequately grazed, and is thus permitted to grow mature, will botanical factors exert a primary influence on the chemical composition and nutritive value.

If the figures in Table XI be compared with the corresponding data obtained in the 1925 experiment, it will be seen that the two types of herbage, despite their similarity in protein content, do actually display, in a consistent manner, certain distinguishing characteristics in respect of chemical composition. These characteristics are brought out in Table XIII.

Table XIII. *Showing differences in chemical composition between the herbage of the 1925 and 1926 pasture plots (data expressed on basis of soil-free dry matter).*

	1925 plot			1926 plot		
	Extreme values		Mean value	Extreme values		Mean value
	%	%		%	%	
Ether extract ...	4.72	to 6.45	5.29	5.71	to 7.41	6.48
Crude fibre ...	12.33	to 17.68	15.39	15.56	to 19.89	18.31
N-free extractives	42.04	to 52.11	44.79	36.77	to 46.49	41.24

It may be concluded from the data in Table XIII that the herbage from the heavy-land pasture was somewhat richer in ether extract and crude fibre, and poorer in carbohydrate, than that from the light-land pasture of 1925. The mineral analyses carried out on the pasture cuts (see Section III) demonstrated further that the 1925 herbage contained a distinctly higher percentage of lime, and a slightly lower percentage of phosphate, than the 1926 herbage. That the differences noted in Table XIII between the amounts of the organic constituents in the two types of herbage were not connected with any fundamental differences in chemical character and digestibility will become apparent when the results of the digestion trials are discussed in Section II.

*Comparison of composition of pasture cuts with that of hay and aftermath cuts.* A comparison of the analytical data for the pasture cuts with those

for the hay cut reveals the effect of advancing maturity on the composition of grass. The dry matter of the hay was appreciably poorer in crude protein, considerably richer in crude fibre and nitrogen-free extractives and, as would be expected, contained a smaller percentage of "amides" than the dry matter of the pasture cuts. In addition, the hay was found to contain a much smaller percentage of lime and phosphate than the pasture herbage (see Section III). The first aftermath cut from the hay plots was taken on July 8, and the stubble-like growth thereby removed resembled the hay in respect of composition. Thereafter, under the influence of a system of weekly cuts, the composition of the successive aftermath cuts improved progressively, until it equalled that of closely grazed pasture, not only in respect of organic composition, but also of mineral content (see Section III).

The foregoing observations are in entire harmony with the findings of the 1925 investigation in this connection and serve to demonstrate that, from the analytical standpoint, the aftermath from a crop of hay, provided the hay has been shorn off close to the ground, has a feeding value comparable to that of closely grazed pasturage. Agriculturists frequently assign a comparatively low value to the aftermath from a hayfield; probably this is largely due to the fact that much stubble is left by the grass cutter, especially when the crop is laid, and this stubble is not only of low digestibility and nutritive value, but prejudices the composition of the aftermath growth. The results of this work would indicate the importance of paying great attention to the close cutting of grass when making hay, in order that the high quality of the aftermath may be assured.

In Table XIV are given the results of an analysis of an aftermath

Table XIV. *Composition of single aftermath cut taken on October 19.*

				%
Crude protein ...	...	...	...	12.41
Ether extract ...	...	...	...	3.96
N-free extractives ...	...	...	...	55.52
Crude fibre ...	...	...	...	23.18
Ash ...	...	...	...	4.93
True protein ...	...	...	...	10.33
"Amides" ...	...	...	...	2.08

cut which was made on October 19 from a separate plot which had remained untouched from the time of haymaking in June.

The plot yielded at the rate of 2800 lb. of dry matter per acre, a much bigger yield than was obtained from a corresponding plot in the 1925 investigation. The explanation of this difference lay in the fact

that the stubble was removed from the 1925 plot by light grazing after completion of haymaking operations, whereas that on the present plot was left untouched. This different treatment was further reflected in the figures for the composition of the 1925 and 1926 single aftermath cuts, the data in the present case lying much closer to those for hay than was the case in the previous year. The results in this respect emphasise what has already been written in regard to the desirability of carefully cutting the grass close to the ground during haymaking, in order that the subsequent aftermath growth may attain to the protein-rich character of young pasture herbage.

## SECTION II. SEASONAL VARIATIONS IN THE NUTRITIVE VALUE OF THE PASTURE CUTS.

No attempt will be made here to give a complete account of the conditions under which the digestion trials were carried out. These have been described very fully in the first communication, to which the reader is referred for detailed information under this heading.

The pasture-grass sample obtained from the cutting of a sub-plot was, after transport to the metabolism building, spread out in a thin layer on a stone floor and left overnight. Under these conditions, no heating of the material occurred. On the following morning, the mass of herbage was thoroughly mixed and the feeds for the day were weighed out. The daily ration consisted of 4400 gm. of the grass and was fed in two equal portions during the day. Moisture determinations on the grass as weighed out for the animals were made daily.

Two pure-bred Suffolk wether sheep, aged about 14 months at the commencement of the experiment, were employed for the purpose of the digestion measurements. The experimental periods, during which the excreta were collected and analysed, were of 14 days' duration. On the completion of an experimental period, the animals were taken out of the digestion crates, weighed and allowed a short rest period, during which time they continued to receive the experimental grass ration. Up to the end of period 4, the rest period was of 3 days' duration; thereafter it was lengthened to 7 days. During the rest intervals, the sheep were allowed daily walking exercise in the open air under supervision.

The details in regard to the average daily weights of faeces voided by the sheep during the different periods and the composition of the composite faeces samples are given in Tables XV and XVI. The results of the analysis of the grass samples have already been recorded in Table VIII (Section I).

Table XV. *Mean daily weights of faeces.*

Period	1		2		3		4		5	
Sheep	III	IV	III	IV	III	IV	III	IV	III	IV
Fresh weight (gm.)	645	920	530	732	660	682	631	594	857	681
Dry matter (%)	37.48	26.38	40.05	30.05	31.53	32.18	33.48	35.45	29.40	36.03
% N on fresh faeces	1.198	0.885	1.208	0.936	0.987	0.981	0.999	1.034	0.878	1.058

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Period	6		7		8		9		10	
Sheep	III	IV	III	IV	III	IV	III	IV	III	IV
Fresh weight (gm.)	612	687	599	664	705	740	730	774	1006	1009
Dry matter (%)	39.36	36.74	40.75	35.85	38.77	37.02	44.11	40.12	43.59	39.28
% N on fresh faeces	1.123	1.065	1.156	1.025	1.019	1.008	1.067	1.015	0.919	0.906

Table XVI. *Composition of faeces samples (on basis of dry matter).*

Period	1		2		3		4		5	
Sheep	III	IV	III	IV	III	IV	III	IV	III	IV
	%	%	%	%	%	%	%	%	%	%
Crude protein	20.17	20.41	18.72	18.74	18.80	18.44	17.48	17.56	17.45	17.54
Ether extract	9.92	10.02	8.25	8.78	9.53	9.91	9.75	10.40	11.60	12.33
N-free extractives	31.38	31.21	30.33	31.67	32.03	30.81	35.58	35.18	34.56	33.68
Crude fibre	11.74	12.93	12.20	11.29	11.37	12.80	12.09	12.09	12.94	13.38
Ash	26.79	25.43	30.50	29.52	28.27	28.04	25.10	24.77	23.45	23.07
CaO	4.076	3.556	4.700	4.410	4.922	5.061	4.526	4.623	4.607	4.594
P <sub>2</sub> O <sub>5</sub>	4.311	4.236	4.427	4.393	4.604	4.872	4.314	4.413	4.045	4.211

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Period	6		7		8		9		10	
Sheep	III	IV	III	IV	III	IV	III	IV	III	IV
Crude protein	18.04	17.30	17.82	16.50	17.78	16.23	14.84	15.23	12.90	13.98
Ether extract	12.87	13.03	12.12	11.97	12.22	12.30	9.90	10.81	6.94	7.10
N-free extractives	34.45	34.76	36.27	36.99	34.37	35.32	31.42	31.39	28.93	29.94
Crude fibre	13.86	14.48	13.76	14.53	15.30	15.53	13.50	13.94	12.44	14.55
Ash	20.78	20.43	20.03	20.01	20.33	20.62	30.34	28.63	38.79	34.43
CaO	4.987	4.660	4.667	4.504	4.182	4.231	4.334	4.116	3.933	3.786
P <sub>2</sub> O <sub>5</sub>	4.169	4.329	4.208	4.330	3.772	3.861	4.200	4.342	3.177	3.500

Table XVII. *Mean percentage of dry matter in grass as consumed.*

Period	1	2	3	4	5	6	7	8	9	10
Per cent.	25.10	21.63	23.22	21.64	24.59	23.84	24.09	25.35	26.06	30.26

Table XVIII. *Summary of digestion coefficients  
(mean for two sheep).*

Period	1	2	3	4	5	6	7	8	9	10
Mean date (1926)	Apr. 19	May 6	May 24	June 10	July 1	July 22	Aug. 12	Sept. 2	Sept. 23	Oct. 14
	%	%	%	%	%	%	%	%	%	%
Dry matter	77.4	77.2	79.0	77.8	77.0	76.5	77.2	75.5	72.0	63.7
Organic matter	81.2	81.8	83.1	81.4	80.4	79.2	79.9	78.3	77.6	75.8
Crude protein	82.7	81.8	80.8	82.0	81.5	83.2	84.1	83.4	83.5	80.6
Ether extract	60.6	67.8	63.3	68.1	61.0	58.6	57.2	52.0	56.1	58.6
N-free extractives	82.7	83.0	85.4	80.9	81.0	79.0	78.7	78.3	75.6	76.0
Crude fibre	81.7	82.9	85.3	85.8	83.5	82.1	83.4	80.7	80.2	73.7
Ash	49.0	46.2	45.6	45.9	47.5	52.3	52.4	50.7	33.9	8.9

*Comments on Tables XVII and XVIII.*

It will be noted that the dry matter content of the herbage as fed to the sheep was by no means constant throughout the course of the experiment, the percentage of dry matter varying from 21.63 in period 2 (corresponding with a daily ration of about 950 gm. of dry matter) to 30.26 in period 10 (corresponding with a daily ration of about 1330 gm. of dry matter). Such variations in the bulk of the ration might at first sight be held responsible for the observed variations in digestibility. Abundant evidence was cited in the previous article, however, to show that the digestibility of a ration is only affected in an insignificant degree as the bulk of the ration is increased. Moreover, if the digestibility of a feeding stuff were in any appreciable measure depressed by increasing the bulk of the ration, then, in the present investigation, the digestion coefficients for the grass should be higher in period 2 than in period 3 and very much higher than in period 1. An inspection of the data in Table XVIII shows that this was, however, not the case.

A consideration of the seasonal alterations in the digestibility of the pasture cuts cannot properly be based on the data for dry matter, since the grass samples always contained more or less soil, arising, as has already been described, from the unavoidable inclusion of worm-casts, this feature being more prominent in the early and late periods than during mid-season. It will be necessary therefore to utilise the data for the organic matter of the grass samples for the purposes of the discussion.

In Table XIX, the digestion coefficients of the grass samples are compared with the corresponding data obtained in the 1925 investigation.

Table XIX. *Digestion coefficients of organic matter of pasture cuts.*  
*Comparison of 1925 and 1926 results.*

Period ...	1	2	3	4	5	6	7	8	9	10
	%	%	%	%	%	%	%	%	%	%
1925	83.4	83.6	80.7	77.4	74.0	74.4	75.8	79.7	80.7	76.9
1926	81.2	81.8	83.1	81.4	80.4	79.2	79.9	78.3	77.6	75.8

For reasons which will be made clear later, the results for period 10 will not be taken account of in the immediate discussion which follows. The comments given below refer only to the results obtained in periods 1 to 9.

The figures in Table XIX show very clearly that the conclusions which were drawn about the high degree of digestibility of the herbage from the 1925 pasture apply in an equal degree to the produce from the 1926

pasture. The two types of pasture herbage, differing, as has been shown in an earlier section, very markedly in regard to botanical composition, display great similarity in respect of digestibility. Bearing in mind the fact that the digestion coefficient of the organic matter of linseed cake is 80.1 per cent., it will be noted that up to the end of period 5 (July 8) the 1926 herbage was more digestible than the linseed concentrate. Even during the later half of the season, the digestibility of the grass fell only slightly below that of linseed cake; at every stage, it was markedly higher than the corresponding values for palm kernel cake and meadow hay of the best quality.

The digestion coefficients for the crude protein of the pasture grass ranged in the present investigation from 84.1 per cent. in period 7 to 80.8 in period 3. In the 1925 experiment, the extent of variation was from 85.4 per cent. in period 2 to 76.6 per cent. in period 6. It is evident therefore that the two types of pasture herbage are very similar in regard to the extent to which their protein constituents are digested in the animal organism. This similarity also characterised the protein digestion coefficients as determined by artificial digestion of the grass samples with pepsin-HCl. The results are therefore confirmatory of the earlier conclusions that the protein of closely grazed pasture grass is a nutrient of high digestibility, comparing quite satisfactorily in this respect with the protein of linseed cake and being much superior to that of the best quality of meadow hay.

It has already been pointed out in Section I that the pasture samples from the 1926 field contained, on the whole, a larger percentage of ether extract than the 1925 samples. This distinction was further emphasised by the fact that the digestion coefficients of the ether extract ran somewhat higher in the present trial than in that carried out in 1925. This difference, which is shown in Table XX, was not sufficiently marked, however, to warrant the conclusion that the ether-soluble fractions of the two herbages were essentially of different chemical character.

Table XX. *Digestion coefficients of ether extract of pasture cuts.*  
*Comparison of 1925 and 1926 results.*

Period ...	1	2	3	4	5	6	7	8	9	10
	%	%	%	%	%	%	%	%	%	%
1925	60.5	60.0	53.5	51.2	39.7	47.9	55.1	54.9	60.6	57.8
1926	60.6	67.8	63.3	68.1	61.0	58.6	57.2	52.0	56.1	58.6

Though it appeared to be characteristic of the 1926 pasture that its herbage was somewhat richer in crude fibre than that of the 1925 plot, there was, on the other hand, little difference in the extent to which

the fibrous constituents were digested by the animal. In both trials, the fibre of the herbage was of exceptionally high digestibility, the digestion coefficients in the present trial varying from 85.8 per cent. in period 4 to 80.2 per cent. in period 9, and in the 1925 experiment, from 84.2 per cent. in period 3 to 75.1 per cent. in period 7. Indeed, in some of the periods, the fibre appeared to be somewhat more digestible than the carbohydrate fraction, though whether this was actually the case, or whether it was merely an anomaly arising from experimental errors incidental to the estimation of the fibre content of the finely divided samples of faeces, must for the present remain uncertain. The same feature was also noted in the 1925 experiment and has already been fully discussed in the publication dealing with that investigation.

It is, however, justifiable to assert that the results confirm the earlier finding, namely, that the fibre and carbohydrate of closely-grazed pasture grass are of approximately equal digestibility. Intensive grazing of a pasture prevents the herbage from maturing and the cell walls from undergoing lignification. For this reason, the crude fibre of such young herbage is composed mainly of the simple form of cellulose, unmixed with any significant amount of the less digestible lignocellulose. The simple form of cellulose is obviously a nutrient of high digestibility, and its ready breakdown in the alimentary tract of the animal must result in the nutrients enclosed within the plant cells being made easily accessible to the digestive enzymes, thus leading to a more efficient digestion of such constituents than would be the case with more mature herbage.

*Seasonal changes in the digestibility of the pasture cuts considered in relation to the meteorological records.* The foregoing considerations have brought out the fact that the grass samples from the light-land and heavy-land pastures, while differing materially in botanical composition, have in common the characteristic of high digestibility. It has already been shown in Section I that the course of the seasonal changes in productivity was very different in the two investigations, and an inspection of the data in Table XIX reveals the fact that this difference also extended to the seasonal changes in digestibility.

The distinctive feature of the seasonal variation in digestibility of the herbage from the 1925 sandy pasture lay in the gradual falling off during the droughty mid-season and the subsequent recovery which occurred during late summer and early autumn following the coming of rain. It was shown that any factors which tended to reduce the productivity of the pasture also had the effect of bringing about a decrease of digestibility, and for that reason a distinct correlation



between rainfall and digestibility was traceable during the dry part of the year. As with productivity, or more strictly speaking, as a consequence of the rainfall-productivity relationship, there was a distinct lag between the incidence of rainfall and its influence on digestibility.

It was further predicted that where soil conditions, rainfall distribution and botanical composition conduced to active growth throughout the entire season, it was questionable whether the mid-season falling off of digestibility would be so marked as was noted in the 1926 season. The findings of the present investigation fully confirm this prediction. In a season of ample rainfall, and on a pasture which, on account of soil conditions, did not suffer seriously in short spells of dry weather and where, moreover, the mixture of grasses was such as to favour continuity of active growth throughout the season, the digestibility of the organic matter of the herbage underwent no significant depression right up to the conclusion of period 9 at the end of September, the value remaining at a high level during mid-season, at a time when, during 1925, the digestibility of the herbage from the sandy pasture had fallen temporarily to a lower level. Indeed, the observed reduction of digestibility during October in the present trial may be more apparent than real, since the inclusion of a large amount of soil in the grass samples when cutting during period 10 made accurate sampling of the herbage a matter of difficulty and rendered the results in this period less trustworthy than in the preceding periods. Furthermore, it is possible that a

Table XXI. *Amounts of digestible nutrients in dry matter of pasture grass samples. Summary of starch equivalents and nutritive ratios.*

Period	...	1	2	3	4	5	6	7	8	9	10
		%	%	%	%	%	%	%	%	%	%
Crude protein	...	22.51	20.25	17.57	18.99	19.14	21.09	21.41	20.88	22.42	19.61
Ether extract	...	3.53	4.22	3.61	4.88	4.38	4.34	3.66	3.31	3.78	3.41
N-free extractives	...	34.52	35.69	39.70	33.97	34.19	30.83	30.81	31.25	27.80	32.00
Crude fibre	...	12.71	13.43	15.16	16.60	15.65	15.44	16.19	16.05	15.86	12.95
Organic matter	...	73.27	73.59	76.04	74.44	73.36	71.70	72.07	71.49	69.86	67.97
S.E. per 100 lb. dry matter	...	70.61	71.52	73.12	71.93	70.39	68.56	68.11	66.89	65.63	64.27
Nutritive ratio	...	2.46	2.90	3.59	3.25	3.13	2.67	2.58	2.63	2.33	2.69

small percentage of dead elm and oak leaves, which blew over the sub-plots at this late season, may have been present in the herbage, despite the precautions which were taken to exclude such from the rations fed to the sheep. That such may have been the case is rendered probable by the fact that the reduction of digestibility during period 10 was most marked in the value of the digestion coefficient of the fibre, a further contributory cause being the presence of a small proportion of dead

grass stems in the rations fed during this final period (see Botanical Notes). On the other hand, however, it should be remembered that productivity during October had fallen to a relatively low level as a result of the cold temperatures prevailing during that month, and it is quite possible that the falling off of digestibility was truly a reflection of the decline in productivity of the pasture.

*Comments on Table XXI.*

The figures for the percentages of digestible nutrients in Table XXI have been calculated by combining the results in Table XI for the composition of the soil-free grass samples with those in Table XVIII for the digestion coefficients of the samples. The data therefore refer to the amounts of digestible nutrients in the dry matter of the soil-free grass samples.

It is of primary interest to enquire how far the results recorded in Table XXI are in accord with the conclusions drawn from the 1925 data to the effect that the dry matter of young pasture grass possesses the character of a protein concentrate of high nutritive value, and that under a system of close cropping, this character is retained throughout the entire grazing season. Table XXII has been compiled to bring out the relation between the results of the two investigations.

Table XXII. *Showing a comparison between 1925 and 1926 results (data expressed on basis of dry matter of soil-free grass).*

Period ...	...	1	2	3	4	5	6	7	8	9	10
% digestible protein	{ 1925	16.81	22.52	19.76	19.09	18.69	16.25	18.48	22.14	23.45	22.54
	{ 1926	22.51	20.25	17.57	18.99	19.14	21.09	21.41	20.88	22.42	19.61
% digestible organic matter	{ 1925	75.59	75.18	72.67	69.65	66.89	67.71	68.73	72.29	73.41	68.05
	{ 1926	73.27	73.59	76.04	74.44	73.36	71.70	72.07	71.49	69.86	67.97
Starch equiv. per 100 lb.	{ 1925	73.70	72.86	68.92	65.80	62.08	63.56	65.44	68.64	71.02	65.41
	{ 1926	70.61	71.52	73.12	71.93	70.39	68.56	68.11	66.89	65.63	64.27
Nutritive ratio	{ 1925	3.72	2.52	2.84	2.84	2.72	3.38	2.97	2.42	2.32	2.18
	{ 1926	2.46	2.90	3.59	3.25	3.13	2.67	2.58	2.63	2.33	2.69

An inspection of the comparative data in Table XXII shows that the 1925 conclusions in respect of the nutritive value of closely grazed pasture are very clearly confirmed by the figures obtained in the present investigation. Except for differences in seasonal variation, the values for percentages of digestible organic matter and digestible protein, for starch equivalents and for nutritive ratio are strikingly similar for both types of herbage.

If a more detailed comparison of the results of the two investigations be made, it will be noted that slight, though definite, differences occurred

other than those connected with seasonal variation, such differences probably being characteristic of pastures containing on the one hand an abundance of rye grass and clover, and on the other hand a preponderance of creeping bent. The 1926 herbage contained somewhat higher percentages of digestible ether extract and fibre, but a rather lower percentage of digestible carbohydrate than the 1925 herbage.

In view of what has already been written in connection with seasonal productivity, it is not surprising that the nutritive value of the heavy clay pasturage was maintained substantially at its originally high level throughout the greater part of the season. No marked falling off was noted during mid-season as was found to occur on the light sandy pasture during the spell of summer drought. As was foreshadowed in the earlier communication, no serious diminution of feeding value will take place so long as the conditions remain such as to enable the pasture to continue to display vigorous growth.

*Practical significance of results.* It is necessary to bear in mind that the results of these investigations are only applicable to such pastures as are kept short by being grazed to their fullest capacity. The reader is referred to the first publication for suggestions in regard to stocking and general management which would be of assistance in enabling such conditions to be realised in practice.

At this stage, however, it is important to realise that equally good results from the standpoints of chemical composition, digestibility and nutritive value have been obtained in two different seasons on two widely differing pastures, one of which, on the basis of old theories connecting good nutritive properties of pasture with the presence in abundance of certain esteemed species of grasses, would have been expected to yield much better results than the other. In view of these facts, it is difficult to resist the conclusion that the botanical composition of a pasture is of secondary importance, and that management, involving not only efficient stocking but also adequate manuring to ensure density of herbage and vigour of growth, is the primary factor determining the nutritive value of pasture. Botanically, it is desirable that a sward should contain a number of species of grasses of differing seasons of luxuriance, in order to ensure a succession of vigorous growths from early spring to late autumn. That factor complied with, however, it is merely necessary, in order to secure the best results from the nutritional standpoint, to concentrate on preventing the grasses from growing mature. Should the herbage be permitted to grow long and mature, as under meadow conditions for example, there can be little doubt that the

different species would display differing feeding values, and *under such circumstances* the nutritive value of the herbage would be likely to be conditioned by botanical composition. Under pasture conditions, however, it should be possible to overcome any handicap from the botanical standpoint by combining a system of close grazing with the intelligent use of fertilisers.

In considering the results which have been obtained in the 1925 and 1926 investigations, it is but natural to assume that their practical application lies in the closest and heaviest possible grazing of pastures with a view to securing the maximum advantages. While the practice of close grazing is one which from many points of view should not lightly be discouraged in any way, yet it is by no means certain at present whether the results do indicate, without reservations, the advisability of the heaviest possible stocking of pasture. The severe cutting or grazing of grassland implies above everything the production of a herbage whose dry matter is to be regarded as a protein-rich concentrate of high digestibility and feeding value. Such herbage, however, on account of its extremely narrow nutritive ratio, comparable with that of linseed cake, constitutes an unbalanced feed for most classes of stock, even for milking cows and growing animals, but especially for fattening stock. It has been shown in the first publication that a 12 cwt. cow, yielding 4 gallons of milk per day, will consume, when grazing freely on such pasturage, more than twice the amount of digestible protein necessary for its requirements.

In view of the unbalanced nature of the pasture concentrate, it becomes at least a matter of opinion whether the optimum results with stock are possible on such closely grazed grass, unless, as appears necessary, the animals are receiving *all through the season* some supplementary foods, like maize or other cereals, which are rich in carbohydrate. There can now be no doubt that the correct supplement to young pasture grass is the carbohydrate, and not the protein concentrate, the use of the latter merely serving to unbalance further an already ill-balanced food. Especially when the problem is one of fattening on pasture can there be no doubt about the truth of this assertion.

Might it not be possible, however, by a somewhat less severe system of grazing, to secure the herbage at a slightly more advanced stage of maturity, in which condition, whilst retaining the high digestibility and nutritive value of a concentrate, it will at the same time be better balanced in its content of protein and carbohydrate and be more adapted to meeting, in itself, the requirements of young stock and dairy animals?

Further investigations will be directed towards the solution of this important problem. It should be borne in mind that the stage of the work just completed was purposely designed to bring out the results which would follow from a system of very heavy stocking, such as obtains in the sheep-grazing Romney Marsh country.

On the other hand, if the purpose of the grazier is to secure a protein concentrated food from his pastures, then heavy grazing will enable him to attain this end and will incidentally result in the discouragement of weeds and the spreading of wild white clover, especially on pastures where creeping bent is not the dominant species. The best accompaniment to slagging is undoubtedly close grazing.

A future generation may witness the utilisation of large areas of grassland for the sole purpose of production of protein concentrate. Pastures, having the appearance of vast lawns, will be cut over regularly and frequently throughout the growing season, and the nutritious, protein-rich produce will be preserved for feeding to animals in winter confinement, along with balancing home-grown feeding stuffs like meadow hay, cereals and roots. The mode of preservation may either be artificial drying, followed by pressing into cakes or grinding to the roughly powdered form, or it may consist in ensiling the freshly cut grass. Under such a system, the necessity for importing protein concentrates would largely disappear, these giving way to an ideal pasture concentrate. The economic significance of such a system, especially during war time, scarcely needs emphasising. The utilisation of dried and powdered lawn cuttings in the feeding of poultry is a further practical application of the results of this work which might usefully be borne in mind. It should be mentioned that the possible methods whereby these suggestions may be put on a practical basis are being investigated in this Institute at the present time.

In this connection, it may be recalled that a process has been patented quite recently on the Continent for "pulping out" the nutrient food from the cells of green crops like grass and separating it from the fibrous cell-wall material. The fibre-free pulp is then dried, pressed and made into cakes for winter feeding. It is clear from the results which have been discussed in this and the preceding publication that such an expensive process could be dispensed with in the case of young pasture grass, since the fibre in such fodder is easily and almost wholly digestible and cannot therefore hinder the ready digestion and assimilation of the other food nutrients present in the cells.

*Maintenance Requirements of Adult Sheep.*

The data which were obtained in the 1925 investigation were employed to calculate the maintenance requirements of adult sheep in terms of lb. of starch equivalent. Since the grass ration was not designed to produce much gain in live-weight, and since the sheep were permitted only a limited amount of exercise during the trial, it followed that the digestion results were eminently suited to the purpose of calculating such requirements. The data indicated that the maintenance requirement of a 100 lb. sheep was 1.29 lb. of starch equivalent per day, a value appreciably in excess of the previously accepted figure 0.74 lb., which was based mainly on the results of old German experiments.

It is of interest to determine whether the results of the present series of digestion trials yield a value in harmony with that given above. The following data are necessary for the calculation:

Mean daily consumption of soil-free grass dry matter throughout trial	= 1054 gm.
Mean daily consumption of starch equivalent throughout trial	= 1.60 lb.
Mean daily gain of live-weight by both sheep throughout trial	= 46 gm.
Mean weight of both sheep during trial ... ..	= 124 lb.

It will be seen therefore that a daily ration of 1054 gm. grass dry matter, containing 1.60 lb. of starch equivalent, enables a sheep of 124 lb. live-weight to make a daily increase of about 46 gm. The nature of this small live-weight increase may be ascertained from a study of the nitrogen balance data given in Table XXXI. The mean daily nitrogen balance of both sheep throughout the experiment was + 7.7 gm., corresponding with a daily protein storage of about 48 gm. It is obvious therefore that the small live-weight increase of the sheep was the result of protein storage, in flesh and wool, from a slightly super-maintenance diet unusually rich in digestible protein.

Adopting the method of calculation described in the previous article, it can be demonstrated that the maintenance requirement of a sheep weighing 124 lb. is 1.36 lb. of starch equivalent per day. Calculating further with the help of the expression  $M.R. = kW^{\frac{1}{3}}$  it may be inferred that the corresponding requirement for the 100 lb. sheep is 1.18 lb. of starch equivalent. Averaging this result with the previous year's value, namely 1.29 lb., it may be concluded, on the basis of two years' work with four animals, that the daily maintenance requirement of an adult sheep is about 1.24 lb. of starch equivalent per 100 lb. of live-weight, a result agreeing very closely with the value 1.26 obtained recently by Wood and Capstick by statistical treatment of a large number of data accumulated in digestion trials on sheep(4).

*Seasonal Production of Starch Equivalent from Pasture Plot.*

In Table XXIII are given data in respect of the production of dry matter, starch equivalent and digestible protein from the pasture plot during the several periods of experiment. Side by side with these figures are given the corresponding data obtained in the 1925 trial, since by this means an excellent idea of the relative nutritive values of the two distinct types of pasture may be gained.

Table XXIII. *Production of nutrient matter from the 1925 and 1926 pasture plots.*

Period	Mean date of cutting		Mean dry matter per day per acre		Mean starch equiv. per day per acre		Mean dig. protein per day per acre	
	1925	1926	1925 lb.	1926 lb.	1925 lb.	1926 lb.	1925 lb.	1926 lb.
1	April 24	April 16	26.10	15.51	19.24	10.95	4.39	3.49
2	May 11	May 3	38.18	16.69	27.82	11.94	8.60	3.38
3	May 29	May 21	28.98	15.56	19.97	11.38	5.73	2.73
4	June 15	June 7	16.29	20.39	10.72	14.67	3.11	3.87
5	July 6	June 28	8.61	18.52	5.35	13.04	1.61	3.55
6	July 31	July 19	6.43	24.04	4.09	16.48	1.04	5.07
7	Aug. 21	Aug. 9	10.40	18.77	6.81	12.78	1.92	4.02
8	Sept. 11	Aug. 30	8.24	16.76	5.66	11.21	1.82	3.50
9	Oct. 2	Sept. 20	9.13	17.29	6.48	11.35	2.14	3.68
10	Oct. 29	Oct. 11	6.31	10.83	4.13	6.96	1.42	2.12

*Comments on Table XXIII.*

This table may be regarded as a chart of the seasonal usefulness of the two pastures under investigation. The data bring into prominence the distinguishing characteristics of the two types of grassland from the nutritive standpoint. The light-land pasture of 1925 produced nutrient matter at a high rate during the early part of the season, but during the subsequent months, especially during the dry mid-season, the rate of production fell to a very much lower level. In sharp contrast to this behaviour, the rate of production of nutrient matter on the clay-land pasture of 1926 was fairly uniform throughout the season. No marked peak of productivity was noted during spring; indeed, the most pronounced rise in the rate of growth of nutrient food occurred towards the end of July, the clay pasture at this period of the 1926 season producing starch equivalent and digestible protein at more than four times the rate at which they were produced in the herbage of the sandy pasture during the corresponding period of the 1925 season.

The factors which probably governed these striking differences in productivity have been discussed in detail in Section I. Whether the

explanation is to be sought in the differing conditions in respect of soil, weather or herbage, or in the combined effect of all these factors, must be left for future investigation to decide. Work has already been planned with a view to ascertaining the relative importance of these different factors in bringing about the observed differences.

Meanwhile, however, it may be pointed out that the noted differences are not without important practical significance. The problem of stocking pastures to their fullest capacity is obviously easier of solution, if the grazier can feel assured that the production of food from his fields will proceed at a reasonably uniform rate throughout the season. If, on the one hand, the bulk of the season's herbage is produced during the spring, as was the case on the 1925 pasture, then the full stocking of pastures at this stage must inevitably involve the grazier in considerable difficulty, since sooner or later the grazing must become inadequate for such a head of stock. The only alternative in such a case to supplemental feeding on a large scale would be the selling off of surplus animals, a practice which, if widely adopted, might not be sound economically.

On the other hand, however, where pastures do not materially exhaust their capacities for production by an overwhelming growth of produce during spring, but continue throughout the whole season to yield in a steady manner an average abundance of fodder of almost uniform nutritive value, then the grazier is enabled to stock his fields efficiently at the commencement of the year, confident in the assurance that the time will not arise when the pastures will be unable to afford the necessary sustenance for his animals. It is clear that the problems of grassland management are much simpler to cope with under conditions such as obtained in the 1926 investigation than when the conditions of the 1925 experiment prevail. It is scarcely an overstatement to assert that the most desirable attribute which can characterise a pasture is the capacity to produce, in a season of average weather conditions, a steady and uniform yield of herbage throughout the whole course of the grazing season.

It was shown in the previous publication that from April 13 to November 9, the 1925 pasture plot produced (on the basis of one acre) 3065 lb. of dry matter, equivalent for production purposes to 2124 lb. of starch and containing 614 lb. of digestible protein, almost 22 per cent. of this yield being produced during the short period of active growth from May 11 to May 25. Despite the absence of a spring "zenith period," the figures for the productivity of the 1926 plot compare very favourably with the above results. From March 21 to October 24, the 1926 pasture





Period 6												
Consumed	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
	1049.00	942.53	263.72	77.10	405.97	195.74	106.47	1049.00	942.53	263.72	77.10	405.97
Voided	240.90	190.84	42.94	31.00	82.99	33.39	50.06	252.40	200.84	45.75	32.89	87.73
Digested	808.10	751.69	220.78	46.10	322.98	182.35	56.41	796.60	741.69	217.97	44.21	318.24
Digest. coeff. %	77.04	79.75	83.72	59.80	79.56	82.94	52.98	75.94	78.69	82.65	57.34	78.39
Period 7												
Consumed	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
	1059.00	957.55	269.62	67.78	414.60	205.55	101.45	1059.00	957.55	269.62	67.78	414.60
Voided	244.10	195.21	43.25	29.58	88.54	33.59	48.89	238.10	190.46	42.56	28.50	88.07
Digested	814.90	762.34	226.37	38.20	326.06	171.96	52.56	820.90	767.09	227.06	39.28	326.53
Digest. coeff. %	76.95	79.62	83.96	56.36	78.65	83.66	51.81	77.52	80.11	84.22	57.95	78.76
Period 8												
Consumed	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
	1115.40	1001.74	274.95	69.94	438.35	218.50	113.66	1115.40	1001.74	274.95	69.94	438.35
Voided	273.30	217.74	44.88	33.40	93.93	41.82	55.56	273.90	217.42	46.63	33.69	96.74
Digested	842.10	784.00	230.07	36.54	344.42	176.68	58.10	841.50	784.32	228.32	36.25	341.61
Digest. coeff. %	75.30	78.26	83.68	52.25	78.57	80.86	51.12	75.45	78.29	83.04	51.83	77.93
Period 9												
Consumed	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
	1146.60	997.20	297.08	74.53	406.82	218.77	149.40	1146.60	997.20	297.08	74.53	406.82
Voided	322.00	224.31	48.69	31.88	101.17	43.47	97.69	321.30*	221.60	49.12	33.57	97.47
Digested	824.60	772.89	248.39	42.65	305.65	175.30	51.71	825.30	775.60	247.96	40.96	309.35
Digest. coeff. %	71.92	77.51	83.61	57.22	75.13	80.13	34.61	71.98	77.78	83.47	54.96	76.04
Period 10												
Consumed	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
	1331.40	1091.35	295.57	70.70	511.52	213.56	240.05	1331.40	1091.35	295.57	70.70	511.52
Voided	480.00†	268.41	57.75	30.43	126.86	54.55	211.59	485.80†	259.85	57.13	28.14	118.65
Digested	851.40	822.94	237.82	40.27	384.66	159.01	28.46	845.60	831.50	238.44	42.56	392.87
Digest. coeff. %	63.95	75.41	80.46	56.96	75.20	74.46	11.86	63.51	76.19	80.67	60.20	76.80

\* Includes 310.50 gm. dry matter voided per day + 10.80 gm. ash in soil left in trough.

† Includes 438.50 gm. dry matter voided per day + 41.5 gm. ash in soil left in trough.

‡ Includes 396.30 gm. dry matter voided per day + 89.5 gm. ash in soil left in trough.

NOTE 1: The figures for grass consumption are corrected for food residues where necessary.

NOTE 2: The care of the experimental animals was in the hands of Messrs V. Thurlbourn and C. Bendall.

grew (also on the basis of one acre) 3667 lb. of dry matter, containing 2532 lb. of starch equivalent and 754 lb. of digestible protein.

It has already been shown in Section I that the yield of dry matter in the hay cut from the meadow plots was 5190 lb. per acre, whilst that of the aftermath which was allowed to grow unchecked from the time of haymaking until October 19 amounted to 2800 lb. per acre, making a total yield in hay and aftermath of 7990 lb. of dry matter per acre. On the basis of composition, it may be assumed that the starch equivalent per 100 lb. of dry matter of hay was 33 and that of the aftermath was 42, whilst the digestion coefficients of the crude protein of the hay and aftermath may be taken as 56 and 60 per cent. respectively. It can thus be estimated that the plots under a system of cutting for hay and a single late aftermath produced, during the whole season, about 2880 lb. of starch equivalent containing about 465 lb. of digestible protein per acre.

It is clear therefore that although the hay plots produced considerably more dry matter per acre than did the pasture plot, yet this difference largely disappears when the produce is evaluated in terms of lb. of starch equivalent. It is further noteworthy that the hay plots produced per acre only about two-thirds of the amount of digestible protein obtained in the herbage from the pasture plot. It is only necessary to add that results similar to these were obtained in the 1925 investigation.

### SECTION III. SEASONAL VARIATIONS IN THE MINERAL CONTENT OF THE PASTURE CUTS.

As in the 1925 investigation, and for reasons detailed in the previous publication, the seasonal variations in the mineral composition of the

Table XXIV. *Showing amounts of lime and phosphate in pasture cuts (expressed on basis of dry matter of grass as cut).*

Period	...	...	1	2	3	4	5	6	7	8	9	10
			%	%	%	%	%	%	%	%	%	%
CaO	...	...	1.188	1.240	1.125	1.190	1.203	1.390	1.246	1.252	1.278	1.254
P <sub>2</sub> O <sub>5</sub>	...	...	1.117	1.127	1.076	1.091	1.055	1.118	1.080	1.077	1.110	0.988

pasture cuts were investigated, particular attention being paid to the lime and phosphate content. The results for the percentages of these constituents in the grass as cut and fed to the sheep are given in Table XXIV. These figures are given in terms of soil-free grass in Table XXV, allowance being also made for the small amounts of lime in the soil which was included in the pasture cuts. For the purpose of

bringing out seasonal variation more clearly, the corrected data are put into graphical form in Chart II.

Table XXV. *Showing seasonal variations in amounts of nitrogen, silica-free ash, lime and phosphate in pasture samples (data expressed on basis of dry matter of soil-free grass).*

Period ...	1	2	3	4	5	6	7	8	9	10
Mean date (1926)	April 16	May 3	May 21	June 7	June 28	July 19	Aug. 9	Aug. 30	Sept. 20	Oct. 11
	%	%	%	%	%	%	%	%	%	%
Nitrogen ...	4.355	3.962	3.480	3.705	3.758	4.056	4.074	4.005	4.296	3.892
SiO <sub>2</sub> -free ash ...	8.17	8.34	6.79	6.86	6.90	7.93	8.10	7.32	8.39	8.70
CaO ...	1.182	1.232	1.115	1.185	1.198	1.389	1.246	1.249	1.271	1.233
P <sub>2</sub> O <sub>5</sub> ...	1.141	1.164	1.107	1.114	1.076	1.127	1.080	1.094	1.150	1.081
Ratio CaO to P <sub>2</sub> O <sub>5</sub>	1.036	1.059	1.007	1.064	1.113	1.232	1.154	1.142	1.105	1.141

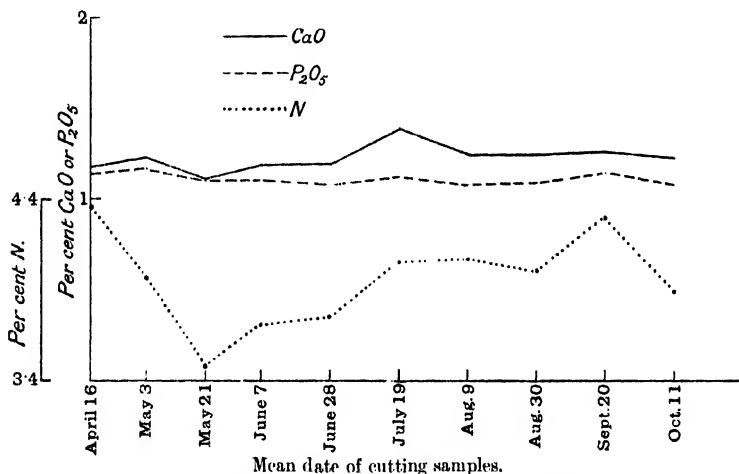


Chart II. Showing seasonal variations in nitrogen, lime and phosphate content of pasture grass (expressed on basis of dry matter).

### Comments on Table XXV and Chart II.

It is interesting to note that the percentage of lime in the 1925 herbage was distinctly higher than the percentage found in the herbage from the present pasture, despite the fact that the 1926 soil contained bigger reserves of lime than did that of the previous year's pasture. On the other hand, however, the 1926 herbage was slightly richer in respect of phosphate than the 1925 herbage. These facts are brought out in Table XXVI and are probably to be attributed partly to the essentially distinct botanical characteristics of the two types of herbage and partly to the fact that the 1925 plot displayed a much more luxuriant development of lime-rich clover than did the pasture in the present trial.

Table XXVI. *Showing comparison of lime and phosphate content of 1925 and 1926 herbage (dry matter basis).*

		Range of variation		Mean value
		%	%	%
CaO	1925	1.230	to 1.809	1.527
	1926	1.115	to 1.389	1.230
P <sub>2</sub> O <sub>5</sub>	1925	0.866	to 1.140	1.035
	1926	1.076	to 1.164	1.113
CaO : P <sub>2</sub> O <sub>5</sub>	1925	1.080	to 2.089	1.502
	1926	1.007	to 1.232	1.105

The results of the 1925 investigation were in harmony with work carried out by Cruickshank (5) at Aberdeen in so far as they demonstrated that the percentage of lime in the dry matter of the pasture herbage rose to a maximum during the first half of the season and then fell off gradually during the second half. The present year's results agree with the foregoing conclusion in so far as the highest percentage of lime in the herbage was noted during July. The range of seasonal variation, however, was much more restricted than in 1925, probably as a result of the relatively poor development of wild white clover in the pasture. Moreover, the falling off in the percentage of lime during the second half of the season was not very pronounced. During Periods 3, 4, 5 and 6, the curves for seasonal variation of lime and nitrogen display a rough parallelism, indicating that a rise or fall in the percentage of nitrogen at this time of the season is accompanied by a rise or fall in the lime content. It would seem probable therefore that during those periods the variations in the percentages of these constituents reflected the activity of the lime- and nitrogen-rich clover plant in the plot. During the second half of the season, as was the case in 1925, these lime-nitrogen relationships were not displayed.

In the 1925 investigation, it was found that the phosphate content of the pasture grass displayed an opposite seasonal variation from that of the lime content, though within a much narrower range. In the present experiment, the percentage of phosphate remained almost constant throughout the entire season, the slight variations which did occur appearing to run parallel with the lime variations, a finding in agreement with Cruickshank's conclusions in this respect. There can be little doubt that the seasonal variations in the mineral composition of pasturage may be markedly different in magnitude and character in different pastures and, indeed, in the same pasture in different seasons. The seasonal changes in the botanical composition of the pasture, in particular the growth of wild white clover, and furthermore, any factors

which affect seasonal productivity, such as meteorological conditions, efficiency of grazing, etc., will all exert an influence on the seasonal mineral composition of the herbage.

As would be anticipated from a consideration of the foregoing statements, the value of the lime-phosphate ratio on the 1926 herbage did not vary within the same wide limits as was the case with the 1925 herbage, although the maximum value was again encountered during mid-season. The essential data in this connection are given in Tables XXV and XXVII.

Table XXVII.  $\text{CaO}:\text{P}_2\text{O}_5$  ratios in 1925 and 1926 herbage compared with ratios for ewe's milk,  $\text{Ca}_3(\text{PO}_4)_2$  and bones.

Ewe's milk	$\text{Ca}_3(\text{PO}_4)_2$	Bones	Mean value in periods 1, 2, 3		Mean value in periods 4, 5, 6, 7		Mean value in periods 8, 9, 10	
			1925	1926	1925	1926	1925	1926
0.86	1.183	1.300	1.264	1.034	1.814	1.141	1.322	1.129

It may be inferred from the data in Table XXVII that the herbage from the 1925 pasture was well balanced in respect of lime and phosphate for the purposes of nutrition of adult animals during the spring and autumn, but that during the drougthy summer, the lime was present in excessive amount in relation to the phosphate. During the present season of well-distributed rainfall, the lime-phosphate ratio, whilst never actually attaining to the ideal value of the ratio in bones, did not at any time of the season indicate that the herbage was seriously unsuited, from the standpoint of mineral composition, to the requirements of adult stock. For very young animals, however, it would appear necessary in both cases to supplement the pasture diet with food containing an excess of phosphate over lime, if the ration is to approximate, in regard to its content of lime and phosphate, to the composition of milk. This might be done by the use of cereals or roots as supplements to the pasture herbage in the feeding of young animals. It might be objected, however, that the addition of such carbohydrate-rich foods would widen the nutritive ratio of the ration unduly. This, however, would not be the case, since the nutritive ratio of the pasture herbage was 2.79 (mean for 1925) and 2.82 (mean for 1926) whilst the corresponding value for milk of average composition is 4.2. If, therefore, calculated on the basis of *dry* matter, two parts of pasture grass (dig. protein = 20 per cent.; nutritive ratio = 2.8;  $\text{CaO}$  = 1.230 per cent.;  $\text{P}_2\text{O}_5$  = 1.113 per cent.) be mixed with one part of maize (dig. protein = 8 per cent.; nutritive ratio = 11.3;  $\text{CaO}$  = 0.05 per cent.;  $\text{P}_2\text{O}_5$  = 0.6 per cent.),

then the resulting mixture will possess a nutritive ratio of 4.2 and a lime-phosphate ratio of 0.88, values agreeing closely with those for milk. Such a ration suitable for very young animals would be secured when about nine parts by weight of fresh pasturage were consumed to every one part by weight of maize.

*Mineral content of hay and aftermath cuts.* The essential data in this connection are summarised in Table XXVIII.

Table XXVIII. *Mineral composition of hay and aftermath cuts (on basis of dry matter).*

No. of cut ...	1	2	3	4	5	6, 7,* 8, 9	10, 11,* 12, 13	Single† late after- math Oct. 19
Date ...	June 25	July 8	July 16	July 23	July 30	Aug. 6, 13, 20, 27	Sept. 3, 10, 17, 24	
SiO <sub>2</sub> free ash	2.72	3.14	4.55	5.04	7.11	7.41	7.30	3.45
CaO ...	0.563	0.622	0.929	1.063	1.092	1.113	1.064	0.671
P <sub>2</sub> O <sub>5</sub> ...	0.597	0.669	0.915	1.010	1.090	1.125	1.162	0.730
CaO : P <sub>2</sub> O <sub>5</sub> ratio	0.943	0.929	1.015	1.052	1.002	0.989	0.912	0.920

\* Composite samples made up as indicated (see Table XII).

† See Table XIV

A comparison of the data in Table XXVIII with those in Table XXV shows that the hay and stubble cuts were not only decidedly poorer in nitrogen than the pasture cuts, but also in respect of lime, phosphate and silica-free ash. Under a system of weekly cuts, the aftermath produce from the hay plots grew progressively richer in lime and phosphate, until the mineral composition resembled very closely that of the pasture cuts, this change being accompanied by a corresponding improvement in organic composition (see Table XII). It will be noted, however, that the values of the lime-phosphate ratios of the hay and aftermath cuts are on the whole smaller than the corresponding ratios of the pasture cuts. This fact may be taken as a reflection of the poor growth of wild white clover in the hay plots, a circumstance which has already been commented on in an earlier part of this paper.

*Loss of nitrogen, lime and phosphate from the soil under pasture and meadow conditions.* The question of the best means of increasing the productivity of pastures by the application of fertilisers is receiving a large amount of attention at the present time, and for that reason it is of interest to calculate from the available data the amounts of nitrogen, lime and phosphate contained in the herbage mown from the hay and pasture plots respectively. As this aspect of the problem was not fully dealt with in the previous publication, the corresponding data for the 1925 investigation are also quoted in Table XXIX.

Table XXIX. *Showing amounts of nitrogen, lime and phosphate in herbage per acre under pasture and meadow conditions.*

Per acre	Pasture cuts			Hay cut			Single after-math cut			Total hay and aftermath		
	N lb.	CaO lb.	P <sub>2</sub> O <sub>5</sub> lb.	N lb.	CaO lb.	P <sub>2</sub> O <sub>5</sub> lb.	N lb.	CaO lb.	P <sub>2</sub> O <sub>5</sub> lb.	N lb.	CaO lb.	P <sub>2</sub> O <sub>5</sub> lb.
1925, Apr. 13 to Nov. 9	121	45.6	32.7	76.6	41.1	32.3	31.8	12.4	8.4	108.4	53.5	40.7
1926, Mar. 22 to Oct. 24	146	45.4	40.8	73.4	29.2	31.0	56.0	18.8	20.4	129.4	48.0	51.4

It will be noted from Table XXIX that under pasture conditions distinctly more nitrogen is taken out of the soil per acre in the herbage than under meadow conditions. This does not necessarily signify, however, that the amount of nitrogen lost to the soil is greater under pasture conditions, since a proportion of this constituent is returned to the soil in the excreta of grazing animals. Furthermore, since wild white clover usually flourishes more luxuriantly in the closely cropped pasture than in the meadow, it follows that a fraction of the nitrogen in pasture herbage is derived in the first place from the atmosphere and not the soil.

The amount of lime and phosphate contained in the herbage per acre was in both investigations greater for the hay plots than for the pasture plots. Table XXIX reveals the curious fact that in the case of the 1925 and 1926 pasture plots, despite the marked differences in respect of soil and herbage, the amount of lime removed per acre over the whole season was almost equal in both trials.

By far the most notable feature of Table XXIX is disclosed, however, if the ratio of nitrogen to phosphate for the different types of herbage be calculated. This feature is brought out prominently in Table XXX.

Table XXX. *N : P<sub>2</sub>O<sub>5</sub> ratios in herbage from pasture and meadow plots.*

	Pasture herbage	Hay cut	Single after- math cut
1925	3.70	2.37	3.78
1926	3.58	2.37	2.74

The data in Table XXX disclose the striking fact that the values of the nitrogen-phosphate ratios for the closely grazed, but botanically different, herbage of the two pastures were practically identical. This is further emphasised by the data in Table XXV. The values of the ratio for the different grass samples from Period 1 to Period 10 in both years' investigations lie, with but one or two exceptions, in the neighbourhood of the values shown in Table XXX.



In addition, it will be noted that the nitrogen-phosphate ratio for the two hay cuts, though lower than those for the pasture herbage, were exactly equal.

It is scarcely to be thought that this close agreement is purely coincidental, although its precise significance is not easy to determine on the available evidence. It would appear that the rate of uptake of nitrogen into the plant is intimately connected with, and possibly controlled by, the rate of uptake of phosphate, so that at a given stage of maturity, a definite relation exists between the rates of absorption of these constituents. In the immature plant, as in closely grazed herbage, this ratio displays a maximum value. With advancing maturity, the value of the ratio decreases, owing, it would appear, to a more rapid falling off in the rate of uptake of nitrogen than of phosphate.

The value of the nitrogen-phosphate ratio appears to constitute therefore a definite index of plant maturity and may possibly be considered as a fundamental factor in plant development. This is further brought out by a consideration of the values for the single aftermath cuts given in Table XXX. The 1925 aftermath resembled in composition a pasture rather than a hay cut; the reverse was true of the 1926 aftermath cut. These facts are distinctly reflected in the values of the nitrogen-phosphate ratios for these samples.

Even more striking confirmation is forthcoming in the manner of variation of the nitrogen-phosphate ratio for the hay, stubble and successive weekly aftermath cuts from the main hay plots (see Tables XII and XXVIII). The ratios for the hay and stubble cuts were 2.37 and 2.14 respectively. In the next sample cut from the hay plots, the ratio had risen to 3.19, a value indicating what was actually found to be the case, namely, that the composition of this aftermath cut was no longer similar to that of hay, but approximated to that of a pasture cut. The successive aftermath cuts for the next ten weeks were no longer distinguishable in respect of composition from closely grazed pasturage. The samples, single and composite, analysed over this period displayed nitrogen-phosphate ratios of 3.71, 3.86, 3.87 and 3.73 respectively. It will be noted (Table XXX) that these values would have enabled the pasture-like character of these aftermath cuts to be predicted.

Whether the relationship revealed above signifies that the phosphate constituent of a plant exerts a controlling influence on nitrogenous metabolism, or some phase thereof, or whether it is merely a part of a more general phenomenon respecting a possible control of the general metabolism of plants by the mineral components, are questions which

Table XXXI. *Showing mean daily nitrogen balances.*

Period	N voided per day										Mean daily N balance	
	N consumed per day		Urine		Faeces		Total					
	III	IV	III	IV	III	IV	III	IV	III	IV		
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.		
1	45.77	45.81	30.41	31.64	7.73	8.14	38.14	39.78	+ 7.63	+ 6.03		
2	36.35	36.46	22.06	24.74	6.40	6.85	28.46	31.59	+ 7.89	+ 4.87		
3	34.44	34.51	19.78	22.90	6.52	6.69	26.30	29.59	+ 8.14	+ 4.92		
4	34.45	34.50	19.52	22.88	6.30	6.14	25.82	29.02	+ 8.63	+ 5.48		
5	39.87	39.87	23.97	27.19	7.52	7.20	31.49	34.39	+ 8.38	+ 5.48		
6	42.19	42.19	26.63	28.58	6.87	7.32	33.50	35.90	+ 8.69	+ 6.29		
7	43.14	43.14	28.25	30.57	6.92	6.81	35.17	37.38	+ 7.97	+ 5.76		
8	43.99	43.99	28.26	30.31	7.18	7.46	35.44	37.77	+ 8.55	+ 6.22		
9	47.53	47.53	25.59	26.95	7.79	7.86	33.38	34.81	+ 14.15	+ 12.72		
10	47.29	47.29	27.86	31.27	9.24	9.14	37.10	40.41	+ 10.19	+ 6.88		

Table XXXII. *Showing mean daily lime balances.*

Period	CaO voided per day										Mean daily CaO balance	
	CaO consumed per day		Urine		Faeces		Total					
	III	IV	III	IV	III	IV	III	IV	III	IV		
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.		
1 *	12.795	12.797	0.244	0.939	9.856	8.630	10.100	9.569	+ 2.695	+ 3.228		
2 *	11.812	11.861	0.173	0.313	9.978	9.698	10.151	10.011	+ 1.661	+ 1.850		
3 *	11.580	11.586	0.192	0.215	10.243	11.109	10.435	11.324	+ 1.145	+ 0.262		
4 *	11.337	11.308	0.154	0.198	9.551	9.736	9.705	9.934	+ 1.632	+ 1.434		
5	13.016	13.016	0.143	0.115	11.610	11.274	11.753	11.389	+ 1.263	+ 1.627		
6	14.581	14.581	0.072	0.060	12.014	11.762	12.086	11.822	+ 2.495	+ 2.759		
7	13.195	13.195	0.201	0.183	11.392	10.724	11.593	10.907	+ 1.602	+ 2.288		
8	13.965	13.965	0.141	0.160	11.429	11.589	11.570	11.749	+ 2.395	+ 2.216		
9†	14.654	14.494	0.103	0.100	13.955	12.780	14.058	12.880	+ 0.596	+ 1.614		
10†	16.063	15.293	0.131	0.148	17.246	15.004	17.377	15.152	- 1.314	+ 0.141		

\* Small allowance made for CaO in drinking water. Distilled water used thereafter.

† Allowance made for CaO in soil residues left in feeding troughs.

Table XXXIII. *Showing mean daily phosphate balances.*

Period	P <sub>2</sub> O <sub>5</sub> voided per day										Mean daily P <sub>2</sub> O <sub>5</sub> balance	
	P <sub>2</sub> O <sub>5</sub> consumed per day		Urine		Faeces		Total					
	III	IV	III	IV	III	IV	III	IV	III	IV		
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.		
1	11.990	12.001	0.050	0.049	10.424	10.281	10.474	10.330	+1.516	+1.671		
2	10.681	10.714	0.086	0.077	9.399	9.660	9.485	9.737	+1.196	+0.977		
3	10.956	10.978	0.083	0.072	9.581	10.694	9.664	10.766	+1.292	+0.212		
4	10.358	10.374	0.084	0.076	9.115	9.294	9.199	9.370	+1.159	+1.004		
5	11.415	11.415	0.080	0.089	10.193	10.334	10.273	10.423	+1.142	+0.992		
6	11.728	11.728	0.100	0.087	10.043	10.926	10.143	11.013	+1.585	+0.715		
7	11.437	11.437	0.104	0.072	10.272	10.310	10.376	10.382	+1.061	+1.055		
8	12.013	12.013	0.112	0.084	10.309	10.575	10.421	10.659	+1.592	+1.354		
9*	12.727	12.724	0.084	0.086	13.524	13.482	13.608	13.568	-0.881	-0.844		
10*	13.143	13.130	0.043	0.073	13.931	13.871	13.974	13.944	-0.831	-0.814		

\* Allowance made for P<sub>2</sub>O<sub>5</sub> in soil residues left in feeding troughs.

cannot be answered with any certainty on the basis of the evidence so far obtained. It is intended to make a closer study of such attractive possibilities, bearing in mind the view that the gradual falling off in the value of the nitrogen-phosphate ratio with advancing maturity may be correlated with changes in the proportion of leaf to stem in the herbage. It will be of particular interest, for instance, to ascertain the magnitude of the value and its manner of variation in the plant leaf itself at different stages of growth, and to enquire further into the possibility of similar relationships in connection with the growth and development of the wheat plant.

For the time being, however, it is not unreasonable to suspect that a phosphatic factor may in some way be concerned in the control of the mechanism whereby protein is elaborated in the plant, a circumstance which appears to have received a kind of intuitive recognition in the extensive use which has been made of phosphatic fertilisers in the improvement of grassland.

#### SECTION IV. PROTEIN AND MINERAL METABOLISM IN THE SHEEP DURING THE DIGESTION TRIALS.

The mean daily nitrogen, lime and phosphate balances for the sheep during the several periods of experiment are summarised in Tables XXXI, XXXII and XXXIII.

##### *Comments on Tables XXXI, XXXII and XXXIII.*

*Nitrogenous metabolism.* Throughout the whole trial, both sheep displayed decided positive nitrogen balances, Sheep III uniformly storing nitrogen at a greater rate than Sheep IV, this fact being reflected in the more rapid gain of live-weight by the former animal. It has been shown in Section II that the small live-weight gains of the sheep could entirely be accounted for in terms of protein storage from a slightly super-maintenance diet excessively rich in digestible protein. It is interesting to note that although the sheep were twenty months old at the termination of the trial, neither animal showed any tendency to approach a condition of nitrogenous equilibrium.

*Calcium and phosphorus metabolism.* The figures in Tables XXXII and XXXIII are expressed to the third decimal place in order that the extremely low values for urinary output of lime and phosphate may be compared with greater precision. It is not meant that the resultant

balances should be regarded as possessing significance beyond the first decimal place.

In all the Periods excepting 9 and 10, the slightly super-maintenance ration of grass (4400 gm. per day) more than supplied the maintenance requirements of the animals for lime and phosphate. The sudden change in the character of the balances in Periods 9 and 10 must not be regarded too seriously, since during these final periods the results of the mineral investigation were rendered somewhat less trustworthy owing to the inclusion of appreciable amounts of soil in the grass samples. This not only increased the difficulty of securing representative samples for the determination of the mineral constituents, but also led to the consumption of soil lime and phosphate, a type of constituent probably much less available than the lime and phosphate of the grass. Indeed, it appears probable that the presence of the soil minerals in the ration may have resulted in a serious disturbance of the lime and phosphate metabolism in the animals, and it is therefore advisable to consider only the results up to the end of Period 8.

Table XXXIV. *Mean CaO and P<sub>2</sub>O<sub>5</sub> consumption and balances in Periods 1 to 8 in the 1925 and 1926 investigations.*

		Mean daily consumption		Mean daily balance		Mean CaO : P <sub>2</sub> O <sub>5</sub> ratio	Mean weight of sheep lb.
		CaO gm.	P <sub>2</sub> O <sub>5</sub> gm.	CaO gm.	P <sub>2</sub> O <sub>5</sub> gm.		
1925	{Sheep I	19.4	12.2	+3.2	+1.6	1.56	116
	{Sheep II	19.5	12.2	+3.4	+1.4	—	126
1926	{Sheep III	12.8	11.3	+1.9	+1.3	1.10	125
	{Sheep IV	12.8	11.3	+2.0	+1.0	—	123

The results in Table XXXIV for four different animals display a satisfactory degree of consistency. It will be noted that a sheep weighing about 120 lb. is able to gain lime and phosphate when subsisting on a grass ration containing 12.8 gm. of lime and 11.3 gm. of phosphate. The present experiment, however, is not such as to solve the problem of the minimum daily requirements of such animals for lime and phosphate, since the utilisation of these constituents not only is dependent on the ratio of the amount of the one to the other, but may also be affected by the presence of an excess or deficit of one or more of the other mineral constituents of the ration, and indeed is not independent of the nature and amount of the organic constituents. The final solution of the problems of mineral metabolism can only be arrived at by the employment of a series of rations in which the balance of the minerals has been varied according to a definite plan. Such conclusions as have been obtained

in the present work are valuable in that they have been deduced from the result of long-period experiments and therefore possess a degree of reliability not associated with data accumulated in short periods of experiment.

Before dismissing this phase of the subject, it is of interest to enquire as to how far pasture grass is able, when forming the sole constituent of the diet, to meet the requirements of the dairy cow for lime and phosphate. It may be assumed that a cow yielding 4 gallons of milk per day requires in its daily ration for maintenance and production purposes  $3\frac{1}{2}$  oz. of CaO and  $3\frac{1}{3}$  oz. of  $P_2O_5$ . Such an animal may consume up to 30 lb. of dry matter per day when grazing freely on good pasturage. On the basis of the 1925 data, this amount of herbage would contain about 7.4 oz. of lime and about 5 oz. of phosphate ( $P_2O_5$ ); the same weight of the 1926 herbage would contain 5.9 oz. and 5.3 oz. respectively. It will be seen therefore that the pasture grass in itself can more than satisfy the requirements of dairy cows for lime and phosphate.

#### SUMMARY.

The investigation which has been dealt with in this communication was essentially a continuation of earlier work carried out in 1925 and was primarily designed with the object of ascertaining whether, under greatly differing conditions in respect of soil, herbage and weather, the striking results obtained in the 1925 investigation concerning the chemical composition, digestibility and nutritive value of pasture herbage, under a system of cutting resembling the conditions of close grazing, still held good.

#### *Contrast of conditions in 1925 and 1926 investigations.*

The 1925 pasture was situated on a light sandy soil; the soil of the 1926 pasture was a stiff gault clay.

The meteorological conditions during the 1926 season were markedly different from those of 1925. Not only was rainfall more copious, but it was also better distributed over the season from the point of view of continuity of productivity. The main features of the weather were: an abnormally cold and wet spring; adequate rainfall during mid-season, at a time when droughty conditions prevailed during 1925; a fairly dry autumn; early night frosts in October.

Viewed from the standpoint of Armstrong's early researches into the botanical composition of the herbage on the different types of grassland

in the English Midlands, the herbage of the 1925 pasture, with its abundance of perennial rye grass and wild white clover, would be regarded as being essentially superior, from the nutritive point of view, to that of the 1926 pasture, in which creeping bent was the dominant species, and rye grass and clover were present in relatively small amount.

*Seasonal changes in the botanical composition of the herbage.*

Botanical surveys of the pasture plot were made at six different dates during the period of the experiment with a view to establishing the trend of the botanical changes. The outstanding observation in this connection was the continuous falling off in the amount of grasses like rough-stalked meadow grass and perennial rye grass, the productivity of such species being very adversely affected under the conditions of the experiment, largely owing to the severe competition of the stronger-growing creeping bent, which flourished to such an extent that it constituted quite 90 per cent. of the mown herbage in the later stages of the season. The conditions of the experiment favoured, as has been shown, the development of creeping bent.

Although the frequent and close cutting of the plot undoubtedly exerted a beneficial effect on the development of wild white clover, yet the growth of the latter on the heavy clay pasture was in no sense so luxuriant as was noted on the light sandy pasture in the 1925 experiment, where the improvement effected by the system of cutting was such as might have been produced with the aid of basic slag. Various reasons have been considered to account for this contrasting behaviour, and it appears justifiable to conclude that wild white clover and creeping bent, possibly because of their not dissimilar habits of spreading, do not flourish well together in pastures. For that reason, it might prove a matter of some difficulty to bring about an improvement in respect of wild white clover in pastures where creeping bent is the dominant species; and further, the spreading of creeping bent in a good pasture may lead to a falling off in the amount of wild white clover.

*Seasonal variations in yield and chemical composition.*

The curve for seasonal productivity of the 1926 pasture differed strikingly from that for 1925 in that it exhibited no spring maximum. Although definite spurts in growth of herbage were noted at one or two points of the season, these were not manifested during the early part of the year, nor were they at all comparable in magnitude with that associated with the spring flush of 1925. The absence of a spring "zenith

period" was attributable to the abnormally cold and wet weather which persisted right up to the end of May, accentuated further by the fact that a wet clay soil is slower in warming up to the temperature necessary to enable the grasses to display that intensive activity which is potential to them in spring.

During the first half of the season, when rainfall was abundant, changes in productivity appeared to be controlled primarily by the prevailing temperatures, though this effect, for reasons made clear in Section I, only became distinctly traceable towards the end of May. Distinct indications were obtained, especially during the early part of the season, of a lag between the incidence of changes of atmospheric temperature and their observed effects on productivity, manifestly owing to the slowness with which a wet clay soil is heated up. Quite the contrary effect was noted in the early part of the 1925 season during the investigation of the seasonal productivity of the sandy pasture. In this case, the response to temperature changes was much more prompt, a rise in the mean weekly temperature being reflected in an increased yield during the same week.

Although the soil conditions in the present investigation were such as to enable the pasture to withstand the effects of short spells of dry weather, yet from the end of July almost to the end of September, a period of fairly dry weather interrupted by occasional days of heavy rain, a correlation was traceable between rainfall and productivity similar to that which was noted during the droughty mid-season of 1925 on the sandy pasture. Over this period, it was observed that a rise or decrease in the rainfall during any one week led to a rise or fall in the yield during the succeeding week.

Despite the absence of a spring "zenith period," the clay pasture was, over the whole season, more productive than the 1925 pasture. The probable reasons for this have been discussed in Section I.

The results of the present investigation confirm very decisively the conclusions drawn from the results of the 1925 experiment in respect of the composition of closely grazed pasture. The conclusion has been drawn that, irrespective of botanical composition, or the presence of little or much wild white clover, a pasture will yield a herbage whose dry matter will be exceedingly rich in crude protein; and further, under a system of close grazing, that the high content of protein will be maintained throughout the entire season.

The data for the composition of the hay and aftermath cuts from contiguous hay plots afforded confirmation of the 1925 finding that when

the stubble, remaining after taking the hay cut, has been removed, then the subsequent growth, under a system of close cropping, gradually approaches the character of closely grazed pasturage in respect of both organic and mineral composition. The practical significance of this finding in connection with the utilisation of aftermath has been discussed.

*Seasonal changes in nutritive value.*

The conclusions in this connection, despite the marked botanical differences in the herbage, were in perfect agreement with those drawn from the results of the 1925 experiment. The dry matter of closely grazed pasture grass is to be regarded as a protein concentrate of high digestibility and nutritive value. It appears probable that only where the herbage is allowed to grow long and mature, as on poorly grazed pasture or under meadow conditions, will nutritive value be influenced primarily by botanical composition. The adoption of a system of close grazing, combined with the use of suitable fertilisers to ensure density of herbage and vigour of growth, enables any handicap arising from inferior botanical composition to be overcome.

The prediction in the earlier publication to the effect that there need be no serious mid-season falling off in the nutritive value of closely grazed pasturage, when the conditions in respect of soil, herbage and weather combine to enable the pasture to display continuous active growth, has received confirmation in the results of the present trial.

The findings of the present work in regard to the narrow nutritive ratio of closely grazed pasturage have emphasised again the unbalanced nature of a ration which consists wholly of such herbage. When the diet of animals on such pasturage requires supplementing, then carbohydrate and not protein concentrates should be employed for this purpose, this procedure being correct not only for fattening animals, but also for dairy animals and young stock. Indeed, if the optimum results are to be obtained on closely grazed pasture, it appears necessary that all pasturing animals should receive, at all stages of the grazing season, some supplementary food, like maize or other cereals, which are rich in carbohydrate. The desirability of further work on the influence of less severe systems of grazing on the nutritive value and nutritive ratio of pasture herbage has been indicated.

The future utilisation of large areas of grassland solely for the production of an ideal pasture concentrate for winter feeding, along with balancing home-grown foods like roots and cereals, has been foreshadowed.



The results of two years' work with four animals have shown that the daily maintenance requirement of an adult sheep is about 1.24 lb. of starch equivalent per 100 lb. of live-weight, a figure agreeing closely with the value 1.26 obtained recently by Wood and Capstick by statistical treatment of a large number of data accumulated in digestion trials on sheep.

Comparative tables showing the seasonal production of nutrient matter in terms of dry matter, starch equivalent and digestible protein from the 1925 and 1926 pastures have been given, and on the basis of these, certain questions of practical significance have been discussed.

*Seasonal variations in mineral composition.*

The percentage of lime in the 1926 herbage was distinctly lower, and that of phosphate slightly higher, than in the herbage of the 1925 pasture. In respect of seasonal changes, the present year's results agreed with the 1925 findings in that the highest percentage of lime was noted during July. The range of seasonal variation, however, was much more restricted than in 1925. The probable reasons for this have been discussed. The percentage of phosphate remained roughly the same throughout the season, the slight variations which did occur appearing to run parallel with the lime variations.

The value of the lime-phosphate ratio, whilst attaining a maximum during mid-season, did not vary within the same wide limits as with the 1925 herbage. At no time of the season was the pasture diet seriously unsuited, from the point of view of the lime-phosphate ratio, to the mineral requirements of adult animals. For nutrition of very young animals, however, it has been shown that a ration consisting of nine parts by weight of fresh closely grazed pasturage with one part of maize will possess a nutritive ratio of 4.2 and a lime-phosphate ratio of 0.88, values agreeing closely with those for milk.

In view of the present importance of the question of fertilisers for pasture, the amounts of nitrogen, lime and phosphate taken out of the soil per acre of pasture have been calculated, and an interesting nitrogen-phosphate relationship of possible importance has thereby been disclosed.

Data concerning the utilisation of the protein, lime and phosphate constituents of the pasture grass by the sheep in the digestion trials have been summarised and discussed. The ability of closely grazed pasturage, when forming the sole constituent of the diet, to supply the

requirements of dairy cows for lime and phosphate has been demonstrated.

In conclusion, the writers gratefully acknowledge the generous assistance accorded to them by Mr S. F. Armstrong, M.A. in connection with the botanical side of the work. Thanks are due to Mr D. B. Norman, B.A., Dip. Agri. (Cantab.) who rendered material assistance in the concluding stages of the experiment, and to Mr C. Leaf, B.A. for supplying the weather records. To Prof. T. B. Wood, F.R.S. and to Mr A. Amos, M.A., thanks are also tendered for the continuance of their interest in this series of investigations.

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# STUDIES IN THE PHYSICAL PROPERTIES OF SOILS.

## IV. A FURTHER CONTRIBUTION TO THE THEORY OF CAPILLARY PHENOMENA IN SOIL.

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*(With Ten Text-figures.)*

In a former paper<sup>(1)</sup> of this series certain calculations were presented deriving values for the cohesive forces due to capillarity in a moist soil. Using the simplified conception of the "ideal" soil as one whose particles are uniform spheres in regular packing, approximate expressions for the cohesion produced by water films were obtained in terms of the surface tension, the size of the spheres and the amount of water present. The cohesion varies directly as the surface tension, inversely as the radius of the particles, and it has a certain limiting value for indefinitely small moisture contents which falls slowly as the moisture is increased. The validity of the argument breaks down when the water fills more than about one-quarter of the pore space, owing to the coalescence of the water films into geometrical forms of much greater complexity. For the remainder of the moisture range general considerations were applied which led to the conclusion that the cohesion would increase to a maximum near saturation. Experimental support of this conclusion was put forward based on measurements of cohesion made on moist silt (after ignition).

These formulae were later corrected by Fisher<sup>(2)</sup>, who showed that the effect due to the direct pull of the surface films had been omitted from the calculation, the indirect effect through the pressure deficiency produced in the liquid having alone been considered. Fisher also derived the exact formulae for water films having the shape of the true capillary curve instead of the anchor-ring form which had previously been taken as a sufficient approximation, and he gave detailed tables and diagrams for the errors involved in this approximation. The difference does not rise above about three per cent. so that the physical argument is not materially affected, and the anchor-ring form will be adhered to in what follows. The other corrections also were not such as to change the physical

sense of the conclusions first advanced. Coming to the more general argument, however, Fisher's conclusions diverge widely. The present author's inference of a rise in capillary force as saturation is approached had been based upon the simplest treatment of the soil pores as capillary tubes. Fisher considered that the result arrived at could not be maintained, though he gives no direct reasons for this opinion. He saw no reason to think that the pressure deficiency behind the water films does not fall continuously from complete dryness to saturation. Such a view is necessitated if the successive stages are to be stable, but it led Fisher to conclude from his tables that the pressure deficiency approaches a value at saturation many times less than the value which had been put forward by the present writer. This would require such a low total curvature of the water surface that it is difficult to imagine how such a film could be fitted into the pore space in the later stages of the filling if uniform distribution be maintained. For in these stages the film must enclose only a small volume of air, and this cannot be done by surfaces of low curvature without having large surface areas which would contravene the condition of a minimum surface area which is imposed by surface tension.

In order to clear up this discrepancy it becomes necessary to review the first arguments in greater detail. It will be shown that the difficulty has arisen from considering that the type of water distribution assumed up to the stage of coalescence is unique, whereas there are other stable forms possible over part of this range which give rise to higher values of pressure deficiency. It depends upon the change, already indicated in the first paper, from a type of surface having *opposite* curvatures in the two principal directions and hence a low pressure deficiency, to a type of surface having curvature in the *same* sense in each direction and hence a high pressure deficiency.

In conformity with his view Fisher offered an alternative interpretation of the author's experimental results, suggesting that the force required to produce rupture in a block (which had been the subject of measurement) should be equated to the total work done rather than to the cohesive force. His conclusion as to falling cohesion with increasing moisture could then be reconciled qualitatively with the rising experimental values although no quantitative agreement was proved. It was also pointed out by Fisher that a relatively dry soil is very brittle, that is, rupture of the connecting films occurs for very small displacements between the particles, and this was at once accepted as the probable explanation of the low values of cohesion obtained at low moistures.

A decision as to the other criticisms was left until more conclusive experimental evidence was forthcoming. This has now been obtained by direct measurement of the pressure deficiency, and the results fully confirm the original interpretation of the cohesion measurements. It will be shown, indeed, that this value for saturation which Fisher has called in question, is not only well grounded but is the one which has a predominating influence in determining the capillary behaviour over nearly the whole of the moisture range.

#### THEORETICAL.

In the following paragraphs full use has been made of the contributions to the subject made by several writers, notably Schlichter(3), Wilsdon(4), Keen(5) and Fisher(2). The fresh ground is broken in considering the behaviour after the soil water has coalesced into a continuous body. In the ideal case the effect of gravity will be neglected, and it will suffice to remark that this becomes the more justifiable the smaller the size of the particles.

The main feature with regard to the pore space in the ideal soil which needs to be clearly realised is its essentially cellular nature. In the first place the consideration will be confined to the case of spheres in close

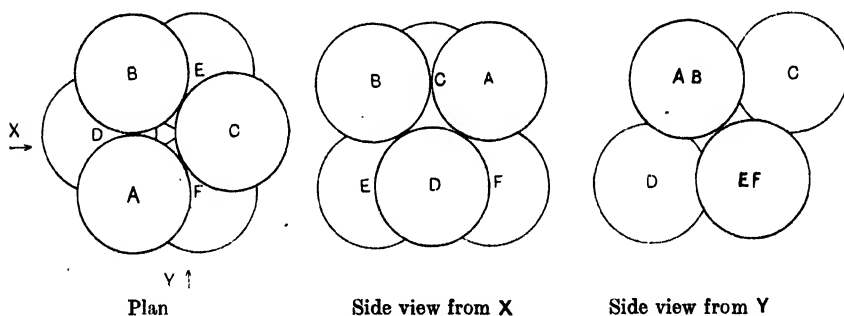


Fig. 1. Six spheres enclosing rhomboidal pore.

or tetrahedral packing, since the reasoning is similar for open packing and may easily be modified to suit this simpler case. Close packing gives rise to a pore space consisting of adjoining cells of two kinds: a smaller kind having a tetrahedral form, and a larger having a cuboidal form. For the latter the more general term "rhomboidal" has been used by previous writers and this term will be retained. The first kind is enclosed by four spheres packed in the form of a pyramid in a fashion which is sufficiently simple to visualise without the aid of a diagram. The more difficult case of the rhomboidal cell enclosed by six spheres is depicted

in Fig. 1, which gives three views of the assemblage from directions at right angles. The continuous pore space of the ideal soil may be imagined as formed by the joining of the apices of the two kinds of cell, each of the four apices of a tetrahedral cell being joined to an apex of four different rhomboidal cells, while the eight apices of each rhomboidal cell are joined to eight tetrahedral cells. There are thus twice as many tetrahedral cells as rhomboidal. The arrangement is shown in Fig. 2

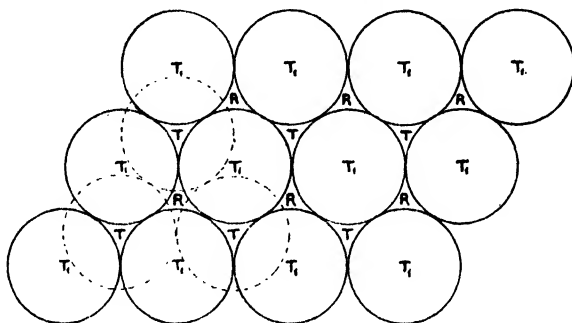


Fig. 2.

which represents a single layer of spheres in close packing. Consider the arrangement of cells made by superposing the next layer above. Of the depressions between the lower spheres those marked with a  $T$  form a socket for a particle in the next layer and so give rise to a tetrahedral cell. In other words the next layer above would be represented by the same series of circles so displaced that their centres came over these pores marked  $T$ . The other depressions marked  $R$  form halves of rhomboidal cells which are completed by the next layer in the manner of Fig. 1. Another series of tetrahedral cells is formed in the centre of the circles at the places marked  $T_1$  (Fig. 2), for here a single sphere in the bottom layer fits into three in the top layer. Thus the rhomboidal (or cuboidal) cell has one apex pointing down to join a  $T$  cell below the first layer, while the opposite apex points up through the top layer. The remaining six apices are grouped in threes at two different levels, one group joining the  $T$  cells marked between the circles of Fig. 2 and the other group joining the  $T_1$  cells marked at the centres of the circles. The shape of the  $T$  and  $T_1$  cells is of course identical.

The matter can be presented in another manner if we examine the shape of the pore space left after the thinner parts round the points of contact of the particles have been filled with water. The water forms rings round the points of contact and these rings can be extended until

they come into contact at their edges. This corresponds to the first stage of coalescence of the water films, and marks the boundary of the range to which mathematical treatment has been applied. A diagram of this stage is shown in Fig. 3 which is a plan of a particle with three of its six neighbours in the same layer. The points of contact with the layer next above are shown by crosses, and the presence of water is indicated by stippling. A particle in the next layer lies symmetrically over the pore on the left, so enclosing a tetrahedral cell, while the pore on the right is covered by three particles in the manner shown in Fig. 1 and a rhomboidal cell is formed. The dry area of each particle is divided into eight portions of triangular symmetry and six portions of quadrangular symmetry, they being so arranged that the apex of a triangle always adjoins the apex of a quadrangle. Six quadrangular areas always appear together so as to form the sides of a rhomboidal cell, and four triangular areas similarly form a tetrahedral cell. Every corner of every cell communicates with the next cell through a sort of tunnel having a circular cross section at its narrowest part of radius  $0.15a$  where  $a$  is the radius of the particles. As previously indicated the communication is always between cells of opposite kinds, and there are twice as many of the small as of the large. The total pore space is divided between water and air in the percentages by volume of 24.3 and 75.7. An estimate indicates that, of the latter figure, about 23.8 is contributed by the tetrahedral cells, and 51.9 by the rhomboidal. This estimate was made by cutting up and weighing a wax cast made with the aid of 2 in. ivory balls.

It is important to note that over the whole of this first stage the shape of the water film is concave in one of its principal directions, and convex in the other, so that the pressure deficiency is produced as the difference between two opposing curvatures. This pressure deficiency is indefinitely great for very small moistures and falls to the value  $4.1 T/a$  ( $T$  being the surface tension and  $a$  the particle radius) at the end of the stage now considered. Over the same range the cohesion values fall from  $4.4 T/a$  to  $3.5 T/a$ , as shown by Fisher(2).

The shape of the remaining air space of the soil at this stage is shown in Fig. 4. The drawing was made from a wax cast which had been pared with a knife to remove the parts occupied by the water rings. The dotted lines represent the outline of the areas where the air is in contact with the soil particles, and the circles which have been removed round each point of contact between particles are easily seen. They are marked with crosses as in Fig. 3. In the centre is shown a rhomboidal cell connected through three of its apices to tetrahedral cells. The remaining apices

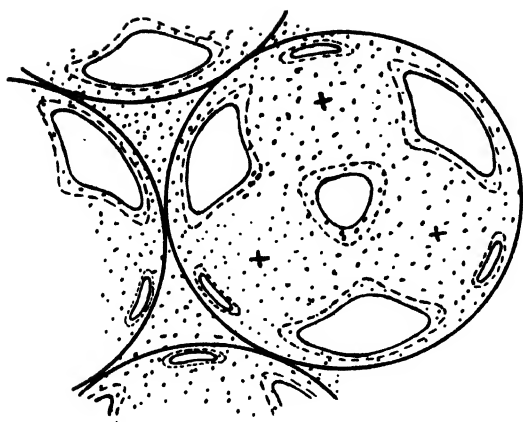


Fig. 5. Distribution of water after final stage of coalescence.

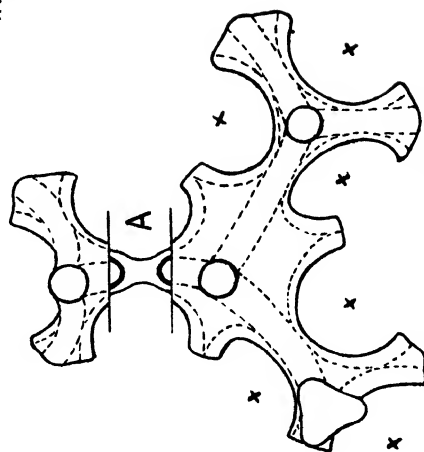


Fig. 4. Perspective drawing showing shape of continuous air space at first stage of coalescence, with development of final stage shown at one neck.

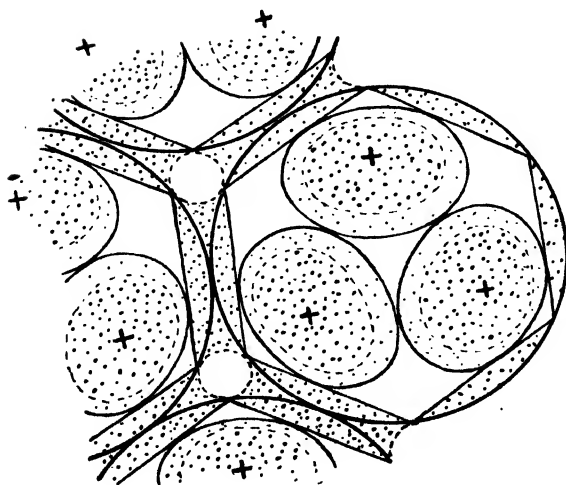


Fig. 3. Distribution of water at first stage of coalescence.



in view have been truncated at the middle of the waist so that the circular section is seen. The continuations from the tetrahedral cells have mostly been truncated somewhat beyond the waist. The interruption in the drawing at *A* is made in order to illustrate later stages in the shape of the films.

Consider now the changes which take place as the volume of water is further increased. The most marked effects will naturally occur where the air-spaces are narrowest, namely at the connecting waists. Here the film draws in, so increasing the concave curvature round the waist. To offset this and maintain a surface of equal total curvature the convex curvature along the waist must also increase. In other words a slight hump is formed which also tends to decrease the size of the waist. Thus each of the anchor rings becomes deformed by a bulge in the three places where it has begun to make contact with others, the effect being to constrict the air-passage joining two cells. This is shown by the thinner lines at *A* in Fig. 4. It is evident that a continuation of this process will quickly close up the waist altogether, and the film suddenly assumes a new shape shown by the thick lines in the same figure the space between the lines now being full of water. It may be remarked in passing that, if the length of a cylindrical film is greater than the circumference of its ends, then it is unstable and the change described takes place spontaneously. The shape of the film at the waist at the first stage of coalescence is well within this definition of stability. This closing of the waists has already been inferred by Keen(5). It may be called the final stage of coalescence. It will be simultaneous through the whole soil, and has the effect of dividing the remaining air into enclosed bubbles of two kinds corresponding to the two kinds of cell. The apices of each cell, instead of communicating with another cell, are closed by a film which is concave in both of its principal directions. This film will therefore have a relatively high pressure deficiency and will retract itself into the more open part of the cell before its total curvature will be reduced to some value near that which it had before the final coalescence. For example, as already shown(1), the values in the pressure deficiency formula at the first stage of coalescence are

$$P = [T/a] (6.4 - 2.3) = 4.1 T/a$$

while a concave film covering the entry to a waist and pushed to the narrowest part might have a pressure deficiency approaching the value

$$P = [T/a] (6.4 + 6.4) = 12.8 T/a.$$

The difference between these two values is so large that there is obviously

a considerable film movement in the readjustment which follows the final coalescence. The water required to fill the waist will be supplied by a retraction of the films back towards the points of contact of the particles. Hence the inference advanced in the first paper as to an abrupt rise in the pressure deficiency at this point seems to be justified. The change would be smoothed, of course, if slight inequalities be supposed to exist in the size of particles or in the packing, so that the waists, being of different sizes, fill up in sequence. An abrupt rise would not contradict the law of conservation of energy since the change is accompanied by a liberation of energy consequent on a diminution in surface area.

The last value  $12.8 T/a$  represents approximately the pressure required to blow a film from one cell through the waist into the next to re-establish communication. It is the value previously put forward<sup>(1)</sup> as the maximum value of the pressure deficiency at saturation, since it represents the suction value required to cause the first entry of the air-water film into a saturated soil. As it is an important critical value it will be called the entry pressure<sup>1</sup>.

In Fig. 5 the case of Fig. 3 is redrawn in its changed aspect when the final stage of coalescence has been completed.

It thus becomes clear that even during the first stage which has been viewed as characterised by isolated annular rings of water, there can exist alternative forms of water distribution after the pressure deficiency has fallen below the value  $12.8 T/a$ , in which the isolation is no longer maintained. Reference to Fisher's tables shows this to be the case for rings whose radii subtend angles between about  $20^\circ$  and  $30^\circ$  at the centre of the particle.

If the water at this stage is introduced so as to close the pore space at a waist then the closure remains, since it would require a pressure equal to the entry value to pull the passage open again. If, however, by some means such as an air blast properly directed the passage were forced open it would remain open, and the annular rings would become isolated in the process and arrange themselves symmetrically, with a drop in pressure deficiency. In fact this is a stage over which the pressure deficiency can be maintained at a constant value in spite of the addition of more water, if only it be added in such a way as to close progressively the connecting waists. This simply depends upon the configuration of the pore space at the waist being such that over the range indicated

<sup>1</sup> The same conception has been employed by H. Bechhold (*Zeit. f. Phys. Chemie*, **64**, 1908, p. 328) in measuring the pore size of ultra-filters. In one method the determination is made by measuring the pressure required to blow air bubbles through the wet filter.

above there are two possible stable positions for a film of given pressure deficiency, one forming an open tunnel and the other closing it with a concave film at each end. Referring to Curve 1, Fig. 6, which shows a graph of the pressure deficiency,  $ABC$  is the curve for the values up to the first stage of coalescence. After any point below  $B$  is reached, however, the pressure deficiency could be adjusted to some value between the lines  $BC$  and  $BD$  by merely manipulating the water films

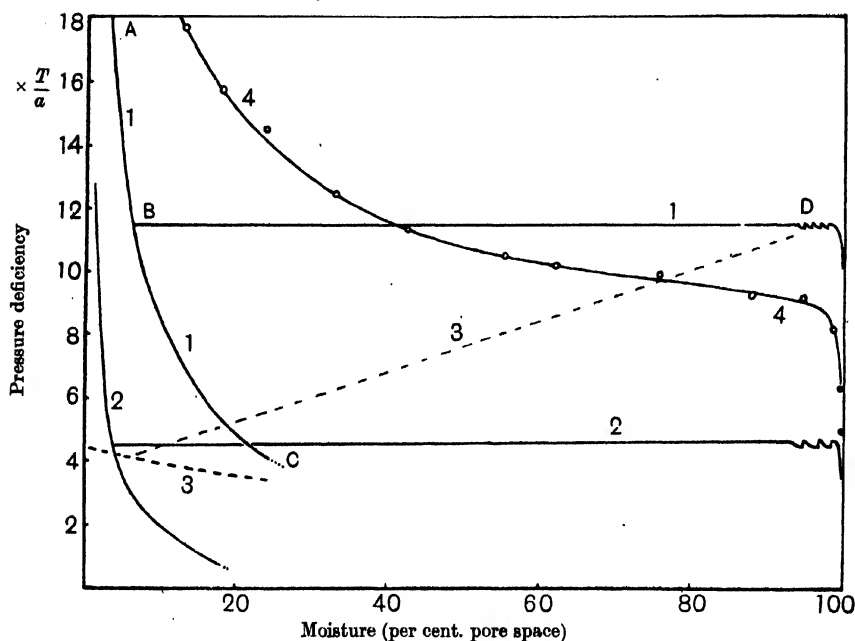


Fig. 6. 1. Pressure deficiency, close packing; 2. Pressure deficiency, open packing; 3. Cohesion, close packing; 4. Experimental pressure deficiency, sand.

in such a way as to close up a sufficient number of the waists, which has the effect of tightening the film in other places. Converting the moistures to a weight basis as used by Wilsdon and by Keen we may say that the type of distribution which has generally been supposed to hold over a range of soil-moisture values from zero up to 3.5 per cent. is only unique up to about 0.8 per cent., after which alternative forms are possible.

In order that the further progress toward saturation may be followed it is necessary that some fresh convention be made as to the manner in which the trapped air is supposed to be removed. It will be simplest

to suppose that the pressure of the air in the soil remains the same as that outside, in spite of the isolation. Consider in the first place the conditions under which any individual bubble may expand or contract. On expansion the water film retreats into the recesses of the cell and the pressure deficiency rises until the critical entry value is reached. It then blows through the corners and re-establishes communication with the neighbouring cells. Equally when the bubble diminishes it reaches an approximately spherical stage for which the pressure deficiency rises until the same critical value is reached. This value corresponds to a spherical bubble having a radius  $0.15 a$  which is the size at which it can pass through the waist and be completely removed from the cell and from the soil. Between these two extremes there is a certain size of bubble for which the pressure deficiency is a minimum, and there will be presumably different values for the large and small types of cell. The minima cannot be very low, as the largest spherical bubble enclosed in a rhomboidal cell has a radius of  $0.29 a$  and therefore gives rise to a pressure deficiency of  $6.9 T/a$ .

Two bubbles in the same liquid and of the same internal pressure cannot remain in stable equilibrium, since whichever is the larger tends to grow at the expense of the smaller, and the larger the discrepancy in size the greater is this tendency. It cannot therefore be assumed that all the bubbles in the ideal soil will gradually decrease in size together as the water increases, but rather that they collapse entirely one by one beginning with the smaller tetrahedral group. The pressure deficiency will remain during this process in the neighbourhood of the entry value, and will give noticeable jumps at the collapse of each bubble if the volume of the individual cell is not too small in comparison with that of the total soil.

So far only the case for increasing water has been considered. In some respects the converse case of water evaporating from a saturated soil is easier to follow. At first a water film of negligible curvature surrounds the soil mass. As the water diminishes this is drawn into the external pores of the soil and the pressure deficiency rises very sharply until the entry value is reached. The threshold of a cell is then crossed and a bubble blown into it which expands into the recesses until the curvature is such as to correspond nearly to the entry value. There will be a slight release in tension due to the redistribution of the water released from the cell, and the magnitude of the release will depend upon the proportion which the volume of a single cell bears to that of the total pore space. To anticipate the experimental part of this paper

it may be mentioned that this *per saltum* movement can be observed under suitably arranged conditions. The process will continue at an approximately constant pressure deficiency as shown along the curve *DB* in Fig. 6. When the air has penetrated into all the cells there will still be a good proportion of the waist connections intact, since on the average one entry for each cell will suffice. It is through these unbroken connecting links that the water has been able to pass easily through the soil to the surface for evaporation. After all the cells have emptied, however, there will be a rapid break up into the isolated water rings previously considered as the first stage. The pressure deficiency then follows the curve *BA*. Thus the case of annular rings of a less pressure deficiency than the entry value, that is an angular radius greater than  $20^\circ$ , does not arise practically except in the case of their gradual growth from a smaller size through condensation from the vapour phase.

The case for open or cubical packing of the soil particles is easily deduced. There is only one type of cell having six apices and eight faces showing triangular symmetry. The radius of the connecting waists at the first stage of coalescence is larger than in the previous case, being  $0.41 a$  and giving an entry value of  $4.8 T/a$  for the pressure deficiency. The graph for the pressure deficiency is shown in Fig. 6, Curve 2. Coalescence begins for an amount of water filling 18 per cent. of the pore space, at which point the pressure deficiency has fallen to  $0.7 T/a$ . The intermediate stage before final coalescence takes place will be more prolonged owing to the greater width of the waists.

The general course for the stress or cohesion exerted between the soil particles may easily be deduced if it be assumed that the pressure deficiency is the main factor in producing the effect. The cohesion values arrived at by Fisher for the low moisture stage and close packing vary only slightly above and below a mean of about  $4 T/a$ , while it is agreed that the pressure deficiency and cohesion are equal for the saturation case since the water film then encloses the soil mass and subjects it to a simple pressure. Thus the cohesion should reach the magnitude of the entry value of  $12 T/a$  at saturation. It must therefore fall between this stage and lower moistures although its exact course is not yet specified. This general conclusion also appears to be warranted when we consider that, while the pressure deficiency remains constant over a considerable range, the soil area over which it is applied diminishes continuously as water is displaced by air in the soil pores. Thus the total stress should diminish also. These inferences are shown graphically in Fig. 6 by Curve 3 drawn in broken line. It may be noted that some of

Atterberg's cohesion curves for sandy soils agree precisely with this theoretical form.

The measurements of Atterberg<sup>(6)</sup> on the cohesion of soils can be interpreted more clearly than hitherto in the light of the above conclusions. This worker made many measurements of soil cohesion at various moistures, and considered that the curves were always broken into two stages. As a wet soil dries out there is a more or less prolonged saturation stage during which the water loss is made up by volume shrinkage. When the shrinkage stops the water films enter the soil in the manner outlined above and there is a consequent distinct lightening in colour. At this point Atterberg found that there was a break or halt in the general trend of rising cohesion with diminishing moisture. Although these breaks on many of his curves are so small as to be within the margin of experimental error—and this has always been the case with measurements made at Rothamsted<sup>(7)</sup>—yet in some cases of heavy soils Atterberg's curves show the break in a most definite manner. If now the total cohesion of a soil be supposed to consist of the sum of two factors, one the usual surface-to-surface molecular attraction and the other the capillary effect, then the flattening of the cohesion curve is simply explained by the falling off in the capillary factor after the entry point has been reached. The distinctness of the break will depend upon the ratio of magnitude of the two factors. From the experimental data for actual soils it would appear that the capillary factor is usually small compared with the other, the exceptions being the cases of sand and of that type of clay which has feeble colloidal properties (*e.g.* kaolin). As evaporation proceeds the rise of pressure deficiency and cohesion from the zero value at complete saturation is not so abrupt in the real case as in the ideal owing to the shrinkage which allows the maximum or entry value to be attained by degrees. This division of the cohesion into two stages, one governed by the capillary action of water films in the grosser pores of the soil and the other by adhesion of colloidal material, was discussed in a former paper<sup>(7)</sup> and seems to be not without theoretical justification.

The question of the capillary rise of water in a soil above a free-water level is one that has received much attention. It will be useful to point out the bearing of the present line of argument upon this subject. So long as the body of water in the soil is continuous the falling off in pressure due to gravitational head of liquid will be proportional to the height above the free-water level. When equilibrium conditions have been reached the gravitational pull at any point is balanced by the capillary

pressure deficiency, and this determines the shape of the film and the distribution of moisture with height. It is therefore clear that the curve already considered for pressure deficiency plotted against proportion of pore space filled with water will give the exact form of the curve obtained by plotting height above free water against moisture content of the soil column. Thus for the ideal case, if the equilibrium be attained by drainage from saturation, the Curve 1 in Fig. 6 shows that the final position is a simple air-water boundary at a height above the free-water level determined by the entry value, with the soil saturated below this boundary. If, on the other hand, equilibrium be attained through capillary rise, then the height of saturation is not so great. The rise of the water front will be checked by the widest section of the pore whereas in drainage the check is in the narrowest section. This will limit the height of saturation in the one case to about half that in the other. In the drainage case, however, the change at the limit of saturation is more abrupt. Above it we have the case of water distributed as isolated annular rings. These attain equilibrium with each other through the vapour phase in the soil atmosphere. The change of pressure with height is therefore much slower than in the case of continuous water films (density of air compared with water). Hence there is a small water-content above the saturated layer which falls off very slowly with height.

An interesting deduction which follows from the existence of a pressure deficiency in soil at all stages of moisture up to saturation is that a soil can never be in true equilibrium with a saturated atmosphere until it is itself saturated. Theoretically, water will condense from such an atmosphere into any body of water which is bounded by a concave surface, since the vapour pressure of such a surface is lower than a plane one. Linford (8) has quite recently discussed this point fully in its bearing upon the determination of the hygroscopic moisture content of soils. The fact that equilibrium is practically attained between soil and a saturated atmosphere at the so-called hygroscopic point, depends of course on the fact that the vapour pressure difference due to curvature of a surface is very small indeed for any but high curvatures. Thus it may be calculated<sup>1</sup> that the vapour pressure difference due to curvature in a pore of  $1\mu$  radius is only about one part in nine hundred although the difference when the radius is reduced to  $1\mu\mu$  becomes one part in two. It is therefore evident that, although the micropores of a soil may quickly fill by condensation, the larger pores can only do so at a very slow rate indeed, so that a practical equilibrium point is attained while

<sup>1</sup> See, for instance, Poynting and Thomson, *Properties of Matter*, p. 168.

the moisture content is still low. It is also clear that where such small differences in vapour pressure are determining the distillation and the equilibrium point a very small change in other physical factors, such as difference in temperature between soil and surrounding air due to incidence of radiation, will have a great effect upon the hygroscopic values obtained. This is fully borne out by Linford's results and by those obtained at Rothamsted by Puri<sup>1</sup>.

This section may appropriately be closed by a consideration of the modification which is produced in the above ideal conclusions when we come to the real case of granular substances in which the particles are neither uniform, nor perfectly spherical, nor packed in an exact manner. The essentially cellular structure of the pore space will presumably be retained, but the sizes and shapes of the cells and of the narrower connections between them will be more varied. If an entry value be calculated from the mean size of the particles we should expect actual entry to take place for some lower value, since many of the pores will have a larger diameter than that given by the theory of closest packing. On the other hand there will also be pores with a higher entry value than that calculated, due to particles below the average size and to the close packing possible with small particles between large ones. These pores cannot be entered until the pressure deficiency rises. It may be inferred then that if the pressure deficiency in a material like sand be measured the entry value will be below that given by the "ideal" formula applied to the mean particle size, and that the curve thereafter will rise slowly instead of remaining level as in the ideal case. Towards lower moistures the rise will be sharper owing to the proportion of cells much "tighter" than would be inferred from the mean particle size. An experimental curve for sand is shown in Fig. 6 (Curve 4) which confirms these conclusions. The same curve should give the water distribution in a vertical column of sand with a suitable alteration in the scale of ordinates to give the height above a free-water level. The form accords with a large mass of data which have been published, and a fuller discussion follows in the experimental section.

Since the real pressure deficiency curve depends upon the sizes of the pores and of the number present of any one size, we are led to the conception of a size-distribution curve for the pore spaces analogous to the familiar distribution curve of particle size as given by mechanical analysis of soil. The conception is more complicated because two sets of values have to be considered instead of one, namely, there is the volume

<sup>1</sup> *This Journal*, 15, 1925, p. 272.



of each pore cell and the size of the largest entry by which it can be approached, instead of the simple particle diameter. But the pressure deficiency curve can be used to obtain information as to the pore size distribution by dividing into groups covering certain ranges of entry value. If the curve be differentiated we get the proportion of the total pore volume made up by cells having an entry value between  $P$  and  $P + dP$ . Thus flatness of the curve indicates a large group of similar pores (completely flat and completely similar in the ideal case) while steepness indicates only small groups of similar pores. The whole problem of the connection between the size distribution of particles and the size distribution of the pore space to which they give rise when packed in a random fashion is one that is yet far from solution although it is here envisaged. Though greatly complicated by physico-chemical questions it is a problem of the greatest importance in soil physics, since a solution of it would enable some of the capillary properties of a soil to be inferred from a simple mechanical analysis.

#### EXPERIMENTAL.

The question of the "pull" exerted by the soil upon the soil water has received much attention, and many experimental lines of attack have been devised both direct and indirect. Much of this work has developed as an investigation of the osmotic equilibrium between the plant root and the soil water. Shull<sup>(9)</sup> used the membrane of seeds, comparing the moisture absorbed by them from soil and from solutions of known osmotic pressure, while Livingstone<sup>(10)</sup>, Pulling<sup>(11)</sup> and others have investigated the possibilities of various artificial membranes for this work. Among other developments was the use of the artificial root point (also examined by Hardy<sup>(12)</sup>) which measures the moisture taken up from the soil by a pencil of porous material such as unglazed porcelain or various close-grained woods. Such questions of purely capillary equilibria as distinct from osmotic equilibria are simpler to investigate as the latter require semi-permeable membranes while for the former any rigid permeable wall (such as a porous pot) can be used. If a closed porous pot containing water and connected to a simple mercury manometer be buried in the soil then communication between the water in the soil and the water in the pot becomes established, and under equilibrium conditions the manometer reads directly the pressure deficiency in the soil water. This apparatus has been used by Livingstone<sup>(13)</sup> and more recently by Kornev<sup>(14)</sup> and by Joffe and McLean<sup>(15)</sup>. Both of the former two workers used the device for the automatic regula-

tion of soil irrigation. Since any moisture content of the soil has its corresponding pressure deficiency it is only necessary, in order to maintain constant moisture, to feed water to the soil through a number of pots connected to a reservoir kept at a reduced pressure of the required value. If the soil moisture decreases the soil suction increases and water is withdrawn from the pots, while flow in the opposite direction takes place if the soil suction falls below the prearranged value owing to an excess of moisture.

Joffe and McLean propose Kornev's method as a simple measurement of the colloidal properties of the soil. They obtain an equilibrium or maximum suction value when dry soil is placed round the pot and left to draw water from it, and they find a correlation between this value and the content of colloidal material in the soil. The capillary suction pressure of a material approaching colloidal fineness would, of course, far exceed the limits of the apparatus for direct measurement, but it is clear that the method of application leads to suction values which are mainly conditioned by the finer pores of the soil. A porous pot cannot be used to measure suction values against a material of finer texture than itself, since it would have a lower entry value than the surrounding material and would admit air to the manometer before the suction values characteristic of the material were reached. Also an upper limit of one atmosphere pressure reduction cannot easily be exceeded by this type of manometer for the measurement of reduced pressures. It seems desirable that Joffe and McLean's method of application should be more critically examined both as to the definiteness of the equilibrium value and the effect on this of altering the amount of soil and of the amount of water delivered from the pot to the soil for unit drop in pressure.

The investigations to be described here have so far been limited to materials approximating in character to the ideal soil, *i.e.* consisting of grains as uniform and as round as possible. The size of grain is not of particular interest so long as it gives rise to pressures which are easily measurable; if the grains are too large water cannot be retained in the pores against gravitational drainage, while if too small the suction pressure exceeds the practical limits defined above. For the materials chosen the porous pot and manometer have been used in the simplest way, namely, to measure true equilibrium values of pressure deficiency under conditions of uniform moisture distribution. Fig. 7 shows diagrammatically one form of apparatus. The granular material is contained in any convenient receptacle, preferably porous to allow of easy evaporation. A good form of container is one made of fine wire gauze soldered

to a frame of stiff wire. A second porous pot in this position has many advantages, but it absorbs a large quantity of water itself which renders it more difficult to calculate the moisture content of the experimental material from the total weight of the apparatus. The difficulty is increased by the capillary behaviour of the pot itself, for it has its own entry value and holds itself saturated by drawing water from within until the suction rises to this value, when it begins to dry out. The effects due to gravitational head of water can be eliminated by making the apparatus as squat as possible and by taking a zero reading of the manometer for saturation, *i.e.* when the soil is immersed up to the mid-level of the buried pot.

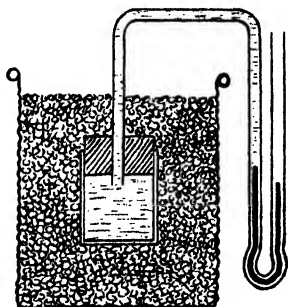


Fig. 7.

With an arrangement such as that shown, the suction or pressure deficiency can be measured from saturation down to low moistures by allowing slow evaporation to take place while at intervals readings are taken of total weight (for moisture determination) and of the manometer height. Each experiment lasted several weeks, and equilibrium was carefully attained before each reading. In order to compare curves for different materials the suction values are reduced to a scale having  $T/a$  as the unit, the value for  $a$  (particle radius) being obtained as a mean of the measurement of a sample of grains under a microscope.

A second method was also used in which each point of the curve was the result of a separate experiment. The manometer pot was rendered impervious with wax except for a small area left as a "window." Over this window the moist material was spread in a layer a few millimetres thick and allowed to dry to any required moisture. The apparatus was then placed in a saturated atmosphere for many hours to attain equilibrium, after which the manometer reading was taken and the moist material scraped off into a weighing bottle for a determination of its moisture. The results were substantially the same as with the other method, which makes it plain that no trouble need be expected from lack of uniformity in packing or water distribution.

A considerable number of materials consisting of approximately spherical grains has been investigated, including shot of various sizes, "glistening dew" (glass spherules), farina (potato starch), sand, silt, and the confection known to our childhood as "hundreds and thousands." The mean particle size ranged from 0.2 cm. diameter in the case of some

shot down to 0.002 cm. in the case of silt. Benzene was used as an alternative liquid to water in cases where solution or chemical action would have taken place. As will be seen from the examples of experimental curves reproduced in Fig. 8 the pressure deficiency in all cases approximates to the form of the ideal case. The entry value is in most cases about one-half that which has been calculated as a maximum for the

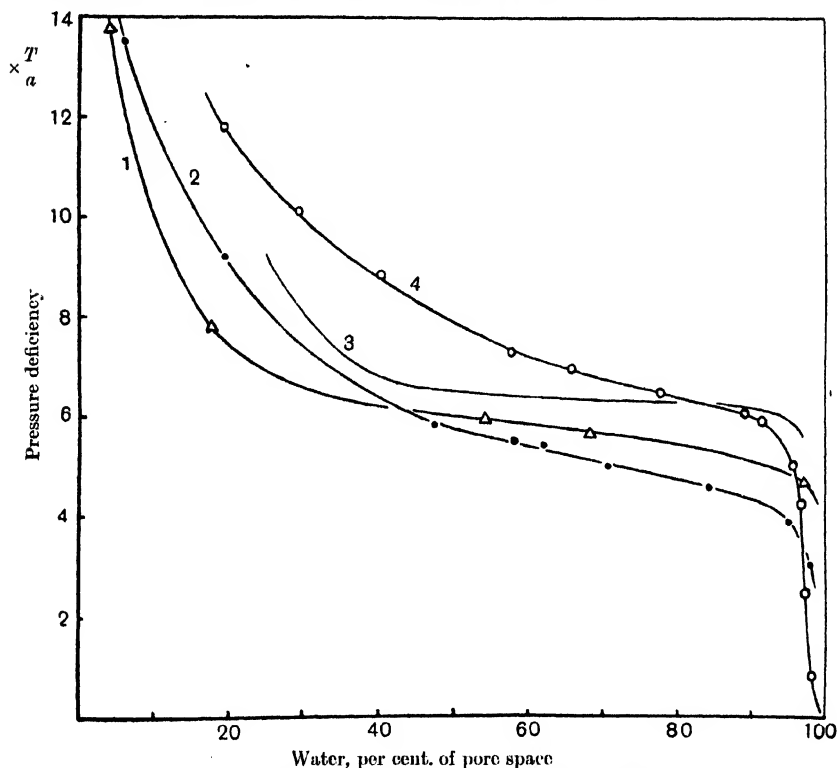


Fig. 8. 1. Sand; 2. "Glistening dew"; 3. "Hundreds and thousands" (benzene instead of water); 4. Ignited silt fraction.

ideal case. But it is to be remembered that that value was an upper limit for the tightest packing, and such a packing is never attained in the real case. Also the experimental entry value depends upon the size of the largest pores presented by the soil, and there will always be many of these larger than the average size calculated from the mean particle radius. A rough comparison may be made on the basis of the total pore space or "porosity" (Buckingham<sup>(16)</sup>). Close and open packing in the ideal cases give a pore space respectively of approximately 26 and

47 per cent. of the total soil volume, and maximum entry values of  $12T/a$  and  $4T/a$  respectively. The total pore space measured for the different materials gave values about 37 or 38<sup>1</sup> per cent. with very few exceptions, *i.e.* about half-way between the two ideal cases. Under these circumstances it is clear that an entry value ranging round  $6T/a$ , as shown in the summarised results of the accompanying table, is a fair

*Summary of Results.*

Nature of grain				Radius cm.	Liquid used	Entry value $\times \frac{T}{a}$	Remarks
Ideal sphere	...	...	$a$	Surface tension $T$ . Water 75, benzene 29 dyne/cm.	4 open packing; 12 close packing	Values are not exact but represent upper limits.	
Sugar pellets (Hundreds and thousands)			·062	Benzene	6·9	Uniformity and sphericity both good.	
Glass spherules (Glistening dew)			·013	Water	4·5	Uniformity and sphericity both good.	
				Benzene	5	Standard deviation of particle diameter 8·5 % of mean.	
Lead shot	...	...	·105	Water	6	Very approximate. Gravitational effect large.	
				Benzene	4		
Round steel grit No. 24			·039	Benzene	11·2	Uniformity poor.	
		40	·018	"	5·7	—	
				Water	7·7	—	
		50	·010	Benzene	5·0	—	
				Water	5·9	—	
Potato starch	...	...	·0026	Water	8·5	Special sample of large grains sepa- rated by sedimenta- tion.	
Sand	...	...	·009	Water	6	Good sample.	
				Benzene	5·8		
Sand	...	...	·016	Water	11	Poor sample.	
					8·5	Same sample im- proved by sifting out finer particles.	
Sand	...	...	·006	Water	5·8	—	
Ignited silt	...	...	·0013	Water	6	—	

verification of the theory already discussed. The values of surface tension taken are those for the pure liquids, and it is probable that contamination caused lower values to rule in most of the experiments, so that the factor 6 errs on the low side. The same value was originally arrived at from the

<sup>1</sup> Mitscherlich records the value 37·2 per cent. for sand in his book *Bodenkunde*, and this value was not appreciably altered by finer grinding.

cohesion measurements on ignited silt. The values inferred by Fisher were to be less than the lowest values for the first stage or calculated case, *i.e.* about  $4.1 T/a$  for close packing and  $.7 T/a$  for open packing. The actual pressure deficiencies represented by the tabulated measurements range from 4.5 to 350 gm./cm.<sup>2</sup>

The main features of the pressure deficiency curve, namely a distinct bend at each end with a flat intervening portion, become smoothed out as the particle size becomes less uniform, for there is then a smooth and wide gradation in the pore sizes. Thus the measurements recorded for actual soils by Kornev show an almost uniform slope for the curve. The converse is well illustrated if the uniformity of a sand is improved by sifting out the finer particles and a comparison made of the suction curves for the material before and after this process. The effect of the sifting may be to lower the entry value, since the small particles decrease the pore size by fitting between the larger ones, but it also has a marked effect in making the middle portion of the curve flatter and the bend at each end much sharper, *i.e.* the ideal curve is more nearly approached.

In regard to the experimental proof of the jumps in pressure which are caused as the air-water surface enters a pore cell and then suddenly expands, it is only necessary to choose a material with large pores and to make the manometric arrangements both sensitive and quick in response. For the latter conditions the inner porous pot must be dispensed with and a mobile liquid chosen, while the manometer tube should be as short and wide as possible but very much narrowed at the point where the liquid meniscus is to be observed. One such actual arrangement consisted of lead shot of about 2 mm. diameter in a small glass vessel filled to the top of the shot with benzene. Dipping into the shot was the open end of an  $\cap$  shaped tube. The benzene was sucked over into the U part of the tube to act as manometer, and the portion of the tube containing the meniscus was of fine quill tubing. As the benzene evaporates and the surface film is drawn down among the shot the manometer meniscus can be seen to give periodic quivers which can be analysed through a telescope as a slow fall (caused by the suction rise to the entry value of a particular pore cell) and a sudden regain (as the film effects entry and the expansion of a bubble to fill the cell gives a momentary release). This expansion of the film in sudden steps can also be observed among the shot through the glass container.

In some results recorded by Deighton<sup>(17)</sup> of experiments on the electrical conductivity of soils there is an apparent confirmation of another of the points brought out by the theoretical discussion. In

working with a sandy soil he found evidence of a metastable state, or alternative values of conductivity at the same moisture, very suggestive of the alternative forms of moisture distribution which have been inferred in this paper for the ideal case. The calculated range lay between 0.8 and 3.5 per cent. moisture for close packing and over a similar range up to 7 per cent. for open packing. Making allowance for hygroscopic moisture, which is almost wholly allied to the colloidal material, Deighton's metastable state was observed over a range of 3 to 4.4 per cent. which is a good agreement considering the probable state of packing. Over this range there was normally a bend in the conductivity curve, but during the first set of readings with increasing moisture the curve continued straight for a distance beyond the bend, and then swung suddenly back to the normal. After wetting and drying again no anomaly was noted. It may well be suggested that the alternative conductivities found corresponded to the alternative forms for the water films. In the position when the waists are closed there is greater film continuity and therefore presumably greater conductivity, and this corresponds to the normal case. The metastable state of unusually low conductivity would then correspond to the circumstance of carrying the moisture value to an unusually high figure without full coalescence of the water films taking place, *i.e.* the "isolated ring" distribution being retained even after the other form is possible (*B* to *C*, Curve 1, Fig. 6). The addition of water in Deighton's experiment was made with warming and mixing, both of which processes would tend to the "isolated ring" form.

It has already been pointed out that the suction curves give the form of the moisture distribution in a vertical soil column which has been allowed to draw up water by capillarity from its base immersed in water. Typical results of such experiments published by Buckingham<sup>(16)</sup> and by MacLaughlin<sup>(18)</sup> have been compared in Fig. 9 with one of the author's suction curves. In each case the material is sand, and although the data for a comparison of particle sizes are not available, the differences in vertical scale shown by the curves may no doubt be attributed to differences in fineness of material. Curve 3 shows the suction curve for a sand (mean particle diameter 0.05 cm., porosity 38 per cent.) in which the ordinates have been simply transposed to the required scale by transforming the suction pressures into inches of head of water. The curve is almost precisely of the form of No. 1 which is the moisture distribution in a sand column as given by Buckingham (porosity 51 per cent.). The falling away at low moisture in his experiment is due to the proximity to the top of the sand column and to the extreme slowness with which

the equilibrium is reached at that stage of low moisture. Curve 2 is a similar moisture distribution found by MacLaughlin which shows a most interesting anomaly, namely a region of maximum moisture occurring above the free-water level. Wadsworth and Smith (19) have also obtained similar results. This receives a clear explanation in the theoretical considerations already advanced. Since this region of maximum moisture occurs at a saturated level it can only be inferred that it is caused by a

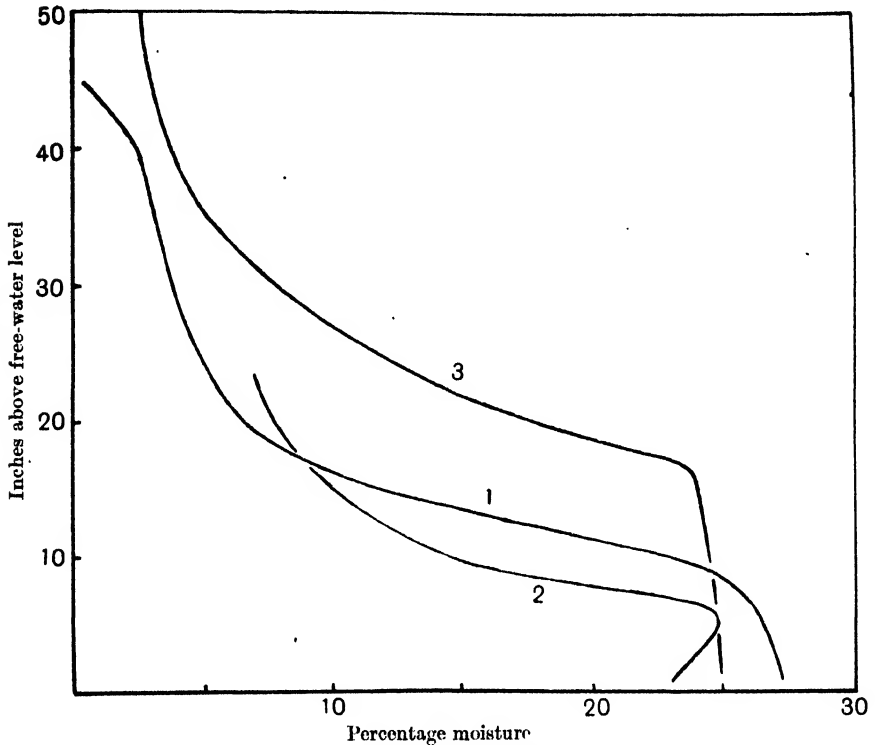


Fig. 9. Distribution of water after capillary rise in sand columns. 1. Buckingham, New Mexico dune sand; 2. MacLaughlin, Idaho sandy soil; 3. Curve inferred from pressure deficiency measurements, sand.

looser state of packing of the particles in the layer concerned, and it is easy to see how this is brought about by the cohesive stress exerted by the advancing water front. It may be assumed that the tightness of packing of the dry soil is never such as to preclude entirely a slight rearrangement when wetted. Then as the water front climbs up through layer after layer it subjects them in turn to a cohesive or shrinkage force, which results in a slightly tighter packing throughout the wetted



material. When equilibrium is reached the total effect of the readjustment will be shown as loose packing in the region of the saturated water front. This is exactly what is shown by the curve illustrated. The soil above this level is subject to less cohesive stress owing to the lower moisture content. The same type of shrinkage under capillary forces is a familiar phenomenon when any loosely packed powder is wetted by a liquid.

A simple experiment was set up with a column of glistening dew in order to test the theoretical deduction that the height to which saturation extends above a free-water level varies according as the equilibrium is reached by drainage or by capillary rise. In the first case the descending air-water surface is checked in the pores at their most restricted cross-section and therefore has a high pressure deficiency, while the ascending film in the second case is checked in the widest section and reaches a lesser height. For the ideal cases in close packing the heights correspond to pressure heads of about  $12 T/a$  for drainage and about  $6 T/a$  for capillary rise. The column of glistening dew was set up in a glass tube and the level to which saturation reached was easily observed through the side. The heights above free-water level which were recorded were  $3.5 T/a$  for drainage and  $1.4 T/a$  for capillary rise. The corresponding value which could be inferred from the direct suction measurement of the entry value was  $4 T/a$  for the drainage case. The rather low value for the capillary rise may be partly accounted for by the effect of repacking discussed in the previous paragraph. With the glistening dew this effect was so marked that an almost complete separation would take place between the contracted wet layer and the dry material above. The difference between the two cases is of the right order and clearly verifies the cellular character of the pore space.

Clay is a material which may be viewed in the same way as these granular materials in as far as it is composed of fine particles, but it has the added property of shrinkage. The entry value or pressure deficiency is slowly approached as evaporation and shrinkage proceed until the point is reached at which simultaneously the shrinkage ceases and entry takes place. The early stages are easily followed using the same apparatus as already described for sand, starting with a very soft plastic clay mass, but in the later stages the suction values rise too high for measurement by such methods. Terzaghi(20) has made such measurements on clays by another line of attack. He subjects the wet clay to large external pressures in a testing machine under conditions allowing seepage, and allows a long period to elapse for equilibrium to be attained. The moisture is then determined. From a series of such measurements he obtains the

external pressure required to reduce the moisture of the clay to any given value. This pressure he considers to be identical with the capillary shrinkage pressure which is present when evaporation has brought the clay to the same moisture. Then if the moisture at the shrinkage limit be known the pressure can be determined which will be required to reduce the moisture to this value, and such a pressure will be the entry value for the clay, or the "transition pressure" as Terzaghi calls it. He calculates this pressure from the particle size on apparently the same theo-

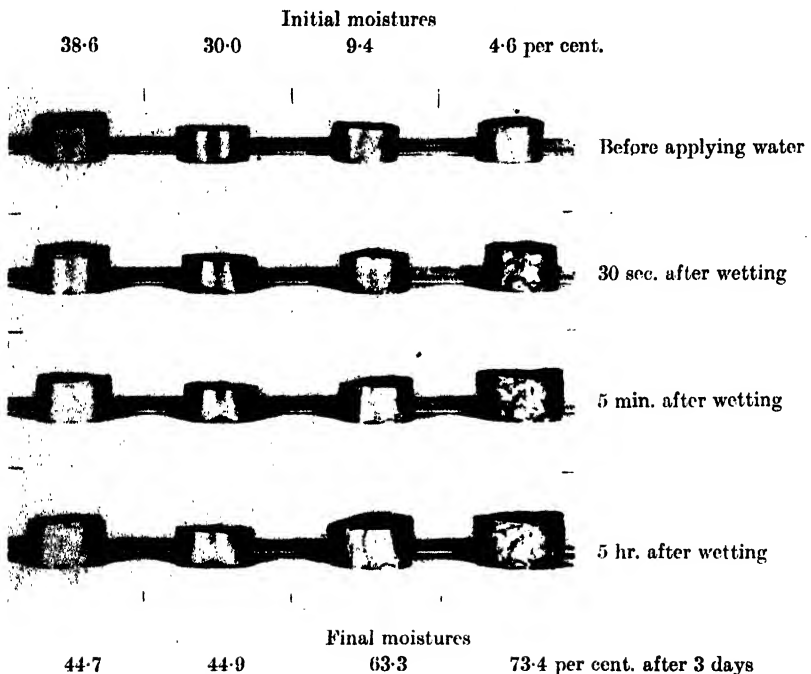


Fig. 10. Showing effect of initial moisture content on the behaviour of clay in capillary absorption of water.

retical basis as used in this paper and obtains values experimentally (of the order  $100 \text{ kg./cm.}^2$ ) which are rather in excess of the theoretical ones.

The converse property to shrinkage, that of swelling when wetted, also gives to clay quite special capillary properties. When clay is saturated in the sense of the pore space being occupied only by water its tendency to swell may still produce a pressure deficiency and enable a further quantity of water to be absorbed. But the process may be slow owing to the minute size and considerable length of the capillaries through which

the water must travel. If, on the other hand, clay is reduced in water content below the saturation point there is a most remarkable change in its behaviour towards wetting. The water can move quickly into the partially empty capillaries since the meniscus at which the surface tension acts is near the water supply and the length of capillary is short. The region so wetted is immediately subjected to the disruptive effect of the swelling of the clay particles, and this, together with the water movement as it is sucked into the clay, causes the clay to crumble to pieces. This in turn makes easy the supply of water to the further regions of the clay and the process continues. If the experiment is suitably arranged the disruption is phenomenally rapid, the crumbling of a mass taking place in a matter of seconds. These properties are illustrated in Fig. 10. A number of similar pellets of wet clay were prepared under pressure in a cylindrical mould. Four of them were brought by evaporation down to the moisture values 38.6, 30.0, 9.4 and 4.6 per cent. respectively. The shrinkage (or saturation) limit for the clay was found to be 15 per cent. by separate experiment<sup>1</sup>. Thus two pellets were above this value and two below. The photographs show the stages in the behaviour of these pellets when they were placed together upon saturated blotting paper from which they could satisfy their capillary attraction for water. As will be seen the two pellets below the saturation value quickly absorb water throughout, accompanied by considerable crumbling. On the other hand the two other pellets show no obvious change, but they manifest the fact that, though "saturated," they are not fully "satisfied" by taking up more water over a course of days. There was no visible change in the state of affairs after the photograph at five hours. The final result is that, owing to the disruption, the pellet which starts with the least moisture ends with the greatest, while those showing no disruption slowly attain an equilibrium value of about 45 per cent. The bearing which these observations have upon the formation of a tilth in the soil under the effect of cultivation operations and weathering is too obvious to need stressing. The "peck of March dust" is certainly an augury of a crumbly seed bed after the next shower of rain, if it may be taken as an indication that the arable soils have been dried out below the entry value of the clay which they contain.

The application of the foregoing remarks, as indeed of the whole matter of this paper, is profoundly modified if the angle of contact between an air-water interface and the soil particle is not very small,

<sup>1</sup> For method of measuring shrinkage see this *Journal*, 13, 1923, p. 296.

i.e. if the soil is not easily wetted. The ease of wetting is an important property to be studied, but its direct measurement by means of the angle of contact would seem to offer great experimental difficulties. In some soils the presence of waxy material or other circumstance may make the angle of contact greater than  $90^\circ$ , when the sign of the capillary pressure is reversed, so that water must be forced into the pores under increased pressure instead of being sucked into them under reduced pressure. Such cases are exceptional and the assumption of a small angle of contact is generally true.

#### SUMMARY.

The theory of the capillary behaviour of moist soil has been further amplified for the ideal case and its relationship to various soil properties considered. Over part of the moisture range which has been dealt with by other authors it is found that there are alternative forms for the water distribution. This appears to explain why some differences of opinion have been expressed regarding some of the main points presented in a previous paper.

The theory is considered in relation to capillary rise in soils as well as to the problem of cohesion previously dealt with. It is shown that the moisture distribution attained by capillary rise can be inferred from simple direct measurement of the suction pressure. Various other experimental illustrations of the theoretical conclusions are introduced.

It has been shown that the suction or pressure deficiency which is necessary to draw an air-water interface into the pores of a soil is one that characterises the capillary behaviour over a considerable moisture range, and the term "entry value" has been applied to it. From a complete suction curve it is possible to infer a size distribution curve for the soil interstices analogous to the treatment of particle size in mechanical analysis of soils.

The results of a number of suction measurements on materials of a granular nature are given.

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MINIMUM MINERAL REQUIREMENTS IN CATTLE<sup>1</sup>.

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In the article "Phosphorus in the Live Stock Industry" (1), published by the present authors in May 1924, a general summary was offered of the relationship between phosphorus deficiency of South African pastures and the measures adopted to obviate the heavy economic losses from specific aphosphorosis in cattle. Since that date further work has been continuously carried out both in relation to the practical possibilities of grading up scrub herds on deficient veld, by combining introduction of pure-bred bulls with rationing on mineral supplements, and in relation to the theoretical physiological minimum mineral requirements of growing stock.

The present paper presents a summary of a preliminary series of orientation experiments, concerning minimum requirements of growing cattle, for calcium, phosphorus, sodium, potassium, and chlorine. Main attention was concentrated upon phosphorus deficiency in the hope of producing clear clinical cases of "aphosphorosis" for subsequent pathological study and comparison with the naturally occurring disease "Styfsiekte," but since the question of "ratio of minerals in dietaries" has attracted so much attention of recent years, this factor is also considered. The vitamine factors come in for incidental review.

No attempt need be made to survey the general literature on mineral metabolism, since the subject has been recently discussed by Orr (2), and a fairly extensive bibliography is readily available amongst the collected papers of the Rowett Institute (3), and in the general review of Shohl (4). In regard to the question of relative proportions of different mineral elements, prevailing views may be roughly grouped as:

(a) The older view that the ratio of mineral constituents in a diet is of little consequence provided the total amount of each essential is adequate; a sort of "Law of the Minimum" applied to animal nutrition.

(b) An extreme modern view that the ratio is of dominant importance and may be responsible for nutritional disorders even if each constituent

<sup>1</sup> Data up to March 1926 presented before the British Association for the Advancement of Science, Oxford, August 1926.

is singly present in sufficient amount. Many of the statements in the recent paper of Marek (5), such as the emphasis upon "Alkali-Alkalizität" and "Erdalkali-Alkalizität," imply this view.

(c) The intermediate view which considers the absolute intake as of primary importance but also emphasises the aspect of optimum relative proportions, and the influence of one mineral constituent upon the absorption and utilisation of another. Orr (2) may be regarded as typifying this view.

Hitherto our own view has been conservative, and while realising the obvious fact that a particular ratio may represent the "optimum" we have rather regarded the current emphasis upon mineral "balance" as exaggerated; and have believed that the physiological capacity of the animal to adjust itself to varying ratios is much greater than credited by many authors. As instances of what we regard as "undue theorising" may be cited the widely quoted work of Ingle (6) in which it is suggested that a faulty ratio of Ca to P is the cause of Osteoporosis in horses in South Africa, whereas in point of fact all the available evidence points to this disease as being of infectious origin; and the recent statement of Marek (*loc. cit.* (5), p. 9) that for Herbivora a strong "Erdalkali-Alkalizität" (*i.e.* preponderance of CaO and MgO over  $P_2O_5$ , on the basis of chemical equivalents) is absolutely *essential*, while for the Carnivora the *reverse* relationship is actually advantageous; a view not suggested by the earlier work of Blatherwick (7) and directly negated by our own experimental data. The physiological problem presented to the herbivora is obviously to get rid of the excess base present in their natural diet, and of the Carnivora or Omnivora to get rid of the excess acid present in theirs, but there seems to be no evidence of any fundamental difference in their physiological mechanisms. A herbivore is obviously a carnivore the moment it is starved, and the reaction of its urine promptly shifts from the alkaline to the acid side according to the base-acid ratio in gross metabolism.

In the experiments now recorded a deliberate attempt was therefore made to subject the cattle to fairly extreme variations of ratios between different minerals in the food, in order to demonstrate the untenability of the extreme views of writers like Marek (5). To do so effectively, meant placing the cattle on the nearest natural approach to a "synthetic ration." It is obviously impracticable to feed batches of cattle on chemically purified food-stuffs, but a fair approach to extreme conditions can readily be obtained by cutting down the "roughage" to the minimum required to obviate digestive disturbance, and meeting the major food

requirements by a "concentrate" such as cereal endosperm. The basal ration in the present experiments, to which various supplements were added to vary the mineral content, was obtained by placing the animals upon a small ration of hay, poor in minerals but otherwise of fair quality, and as much "Fanko" as they would eat. Fanko is a locally produced "cereal flake breakfast food" consisting of the rolled endosperm of maize, of high calorific value and fair protein content, but very low in mineral constituents. During a preparatory dieting period the hay ration was steadily reduced to 2 lb., but as this threatened to produce digestive disturbance it was gradually raised again. At 3½ lb. per head per day no disturbances were noted over several weeks, and this quantity was therefore fixed as sufficiently bulky for the experiment, but as contributing mineral constituents below minimum physiological requirements. To vary the protein moiety of the ration and incidentally ensure sufficient iron, the influence of which was not under consideration, 2 oz. of blood meal were added; an amount leaving other minerals still sufficiently low for the purpose in view. Sampling of the hay and fanko for analysis was conducted by taking a small portion as each day's ration was being weighed out, and mixing to obtain monthly samples. The average mineral content of the various materials used is shown in Table I.

Table I. *Mineral constituents of materials used.*

Material	CaO %	MgO %	K <sub>2</sub> O %	Na <sub>2</sub> O %	P <sub>2</sub> O <sub>5</sub> %	Cl %	SO <sub>3</sub> %
Hay ... ..	0.41	0.33	1.30	0.015	0.17	0.22	0.47
Fanko ... ..	0.01	0.04	0.15	0.017	0.09	0.03	0.14
Blood meal ... ..	0.20	0.02	0.35	1.40	0.55	1.05	0.73
Bran ... ..	0.14	0.50	0.75	0.06	2.10	0.04	0.20
Bone meal ... ..	31.0	0.65	0.10	0.55	22.9	0.11	0.30

From these data the initial basal ration of 3½ lb. hay, 2 oz. blood meal, and 5 lb. fanko works out approximately as follows:

Constituent ... ..	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	Cl	SO <sub>3</sub>
Grams per head per day	6.9	6.5	24	1.4	5.1	4.8	11.1
Milligram equivalents	247	322	508	45	215	135	278

As the experiment proceeded and the animals increased in weight, more fanko was allowed according to appetite, but the hay remained fixed at 3½ lb. The total minerals in the ration, however, are obviously not much influenced as the animals increase their consumption, since even doubling the fanko (to 10 lb.) only increases the total by 0.23 gm. CaO, 0.91 gm. MgO, 3.3 gm. K<sub>2</sub>O, 0.39 gm. Na<sub>2</sub>O, 2.1 gm. P<sub>2</sub>O<sub>5</sub>, 0.68 gm. Cl, and 3.2 gm. SO<sub>3</sub>. From the "milligram equivalents" of total minerals it will be observed that the ration is potentially basic to the extent of



nearly half a litre normal reagent, the term "milligram equivalents" being, of course, the same as "c.c. normal solution." Of this basicity the potassium contributes nearly as much as the other three bases put together, and the sulphur nearly as much as the two other acids together. The Ca and P approximately balance.

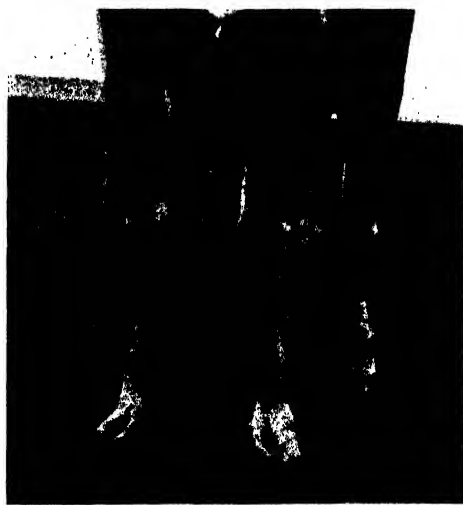
It should be carefully noted at this point that the term "milligram equivalents" is here used in its correct absolute sense, and should not be confused with "mg. equivalents per 100 grams of *dry matter* as used by Marek (*loc. cit.* (5), p. 8). Obviously the term may be used in reference to any arbitrary units; unit weight of food in the form fed, unit grams of dry matter, unit "starch equivalents," unit calories, unit time; or in any other desired fashion. The use of it by Marek on the *dry matter* of the ration, which varies with the *non-digestible* components of the individual foods, will be critically referred to presently. Orr (*loc. cit.* (3)) uses the more legitimate relationship "c.c. *N* solution, or grams mineral constituent, per 1000 caloric portions," and we ourselves (*loc. cit.* (1)) have made use of the ratio " $P_2O_5$  to starch equivalent" in order to indicate that it was not the percentage of phosphorus on the dry matter of the veld vegetation which mattered, but the amount in relation to the energy value of the pasture.

Bearing in mind the nature of the basal ration, the following scheme of eight experiments (duplicate) on sixteen young heifers, to which this short paper is limited, will be clear. Since the experiments were regarded as of a physiological nature reflecting extreme conditions it was not considered essential to secure expensive high-grade animals of "uniform growth curve" and ordinary cross-bred heifers, already available from the laboratory stock, varying in age from 12 to 18 months, were therefore used. It was realised that these might vary considerably in build and potential adult weight, but individuals as similar as possible were picked out from the available selection and arranged in pairs so that the average growth rate of each pair would be sufficiently close for the purpose in view.

*Exp. A.* Low calcium and low phosphorus but adequate in other respects. Basal ration plus rain water, and 25 gm. salt to remove deficiency of Na and Cl. Initial ration therefore  $3\frac{1}{2}$  lb. hay, 5 lb. fanko, 2 oz. blood meal, and 25 gm. sodium chloride. The mineral constituents in the day's food work out at 6.9 gm. CaO, 6.5 gm. MgO, 24 gm.  $K_2O$ , 14.6 gm.  $Na_2O$ , 5.1 gm.  $P_2O_5$ , 20 gm. Cl, and 11.1 gm.  $SO_3$ . The "balance" of bases over acids is obviously not affected by the neutral sodium chloride and is therefore the same as shown for the basal ration, *i.e.*



Photograph 1. Heifer 925 after 15 months on a ration deficient in phosphorus and low in calcium. Shows the stiff gait characteristic of the disease "Styfsiekte."



Photograph 2. Also heifer 925. Shows the malformation of the legs characteristic of phosphorus deficiency disease.

potentially alkaline. There is an absolute deficiency of both  $\text{CaO}$  and  $\text{P}_2\text{O}_5$ , considerably greater than that under which *malnutrition occurs under natural conditions* (*loc. cit.*<sup>(1)</sup>) and which *cannot be remedied* by mere increase of food consumption (*fanko*). There is, however, no shortage of other necessary constituents.

*Exp. B.* Border line in regard to  $\text{CaO}$  and  $\text{P}_2\text{O}_5$ . Same ration as *A*, but supplemented with 25 gm. of bone meal; the usual benzene-extracted steam-sterilised article of commerce, selected because it is the most widely used suitable supplement in South Africa. The 25 gm. of bone meal increases the  $\text{CaO}$  to approximately 15 gm., and the  $\text{P}_2\text{O}_5$  to approximately 11 gm. but, as may be noted from its analysis (Table I) does not materially affect the proportions of other minerals. The ratio of  $\text{CaO}$  to  $\text{MgO}$  is also improved, if that ratio really matters much; a point not under specific investigation. The amounts of  $\text{CaO}$  and  $\text{P}_2\text{O}_5$  were based upon the estimate of the probable natural intake of growing cattle on arid phosphorus-poor soils (Bechuanaland); and regarded as *low* but not necessarily below the physiological minimum for health, although much below the requirements for ultimate milk production.

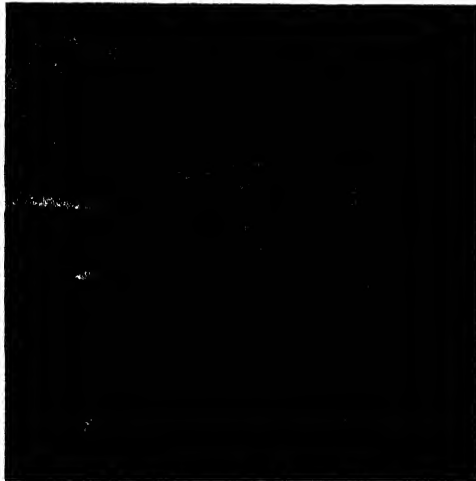
*Exp. C.* Moderate in regard to  $\text{CaO}$  and  $\text{P}_2\text{O}_5$ . Ration same as in *A*, but with the addition of 100 gm. of bone meal, bringing the total  $\text{CaO}$  to 37 gm., and  $\text{P}_2\text{O}_5$  to 28 gm., but again not seriously influencing the proportion of other minerals in *A*. This was regarded as more than sufficient for growth, and indeed sufficient to obviate actual deficiency disease after calving, provided the milk yield is *low*. Since the  $\text{CaO}$  was now high, there was no point in keeping to distilled water as in *Exp. A*, and hence in this experiment, as in all others of high calcium, ordinary tap water was used for drinking purposes.

*Exp. D.*  $\text{CaO}$  high but  $\text{P}_2\text{O}_5$  low. Ration same as in *A*, but with the addition of 40 grams of chalk. This leaves the intake of other minerals as before, but brings the total  $\text{CaO}$  up to about 29 gm., or almost *six times* the amount of  $\text{P}_2\text{O}_5$  (5.1 gm.); 1046 mg. equivalents of  $\text{CaO}$  to 215 mg. equivalents of  $\text{P}_2\text{O}_5$  if phosphoric acid be reckoned as trivalent, or to 129 mg. equivalents if it be reckoned as rather less than divalent (factor 1.8) as it actually is from the point of view of "physiological neutrality regulation."

*Exp. E.*  $\text{CaO}$  low but  $\text{P}_2\text{O}_5$  relatively high. Ration the same as *A*, but with the addition of 2 lb. of wheat bran to supply adequate phosphorus, chiefly as the easily digestible phytin; increasing the  $\text{P}_2\text{O}_5$  by 19 gm. but only increasing other minerals by relatively unimportant amounts, and bringing the total constituents to approximately 8.2 gm.



Photograph 3. Animal 866 on a diet deficient in phosphorus but adequate in calcium, thus establishing Styfsiekte as an aphosphorosis. Shows the painful kneeling position frequently adopted during the month preceding death.



Photograph 4. Abnormal calf of animal 866. Appears to show aphosphorosis at birth.

CaO, 11 gm. MgO, 31 gm.  $K_2O$ , 15 gm.  $Na_2O$ , 24 gm.  $P_2O_5$ , 20 gm. Cl, 13 gm.  $SO_3$ . The ratio of CaO to  $P_2O_5$  is therefore approximately 1 to 3, or in the opposite direction to *Exp. D*, and the whole ration becomes "acidogenic" as the consumption of fanko increases.

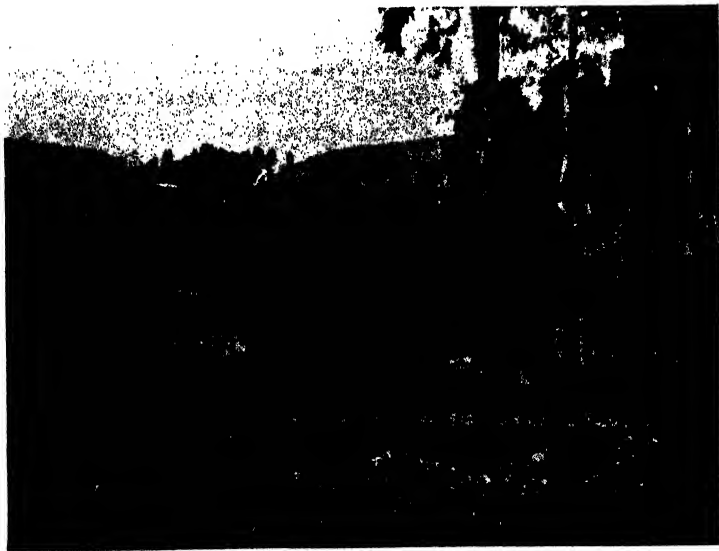
*Exp. F.* Low sodium, low chlorine, but not deficient in other minerals. Ration the same as *A*, but omitting the salt and adding 100 gm. of bone meal to rectify shortage of calcium and phosphorus. The total minerals then work out at approximately 37 gm. CaO, 7 gm. MgO, 24 gm.  $K_2O$ , 2 gm.  $Na_2O$ , 28 gm.  $P_2O_5$ , 5 gm. Cl and 11 gm.  $SO_3$ . Both sodium and chlorine could have been materially reduced by omitting the blood meal, but it was considered best in this preliminary experiment to operate on the border line of deficiency rather than actually attempt to produce a sodium chloride "deficiency disease." It will also be noted that this ration incidentally represents high potassium relatively to sodium.

*Exp. G.* Low sodium but high chlorine and still higher potassium. Ration the same as *F*, but with the addition of 32 gm. of potassium chloride, or the same as *C* with NaCl substituted by an "equivalent amount" of KCl. At a later date (May 1925) the potassium chloride was doubled in order to throw the ratio of  $K_2O$  to  $Na_2O$  still more widely apart. In this experiment the mineral intake is the same as in *Exp. F* except that the chlorine is raised to that of *Exp. C* (20 gm.) but the sodium left low (2 gm.) and the potassium raised to 44 gm.  $K_2O$ , and later to 64 gm.; giving a ratio of over 1 to 20 in the initial stages of the experiment raised to over 1 to 30 in the later stages. Beyond this it was not considered necessary to go, since in any natural vegetation any ratio wider than this is probably *meaningless as a ratio*, and indicative of absolute sodium intake below physiological requirements.

*Exp. H.* Check on the vitamine factor. Ration the same as *C* (i.e. adequate in minerals) but with part of the hay replaced by young fresh green forage. All the diets, *A* to *G*, are obviously deficient in vitamins, with the exception of *Exp. E*, which contains abundance of "water soluble B" in the form of bran. Of the basal ration, the fanko (equivalent to "Samp") contains even less vitamine than polished rice<sup>(10)</sup>, and the blood meal (steam heated and dried in manufacture) very little. The hay (mature) contains a small proportion of vitamins A and B but practically no antiscorbutic vitamine. In any case the small daily intake (3½ lb.) would be quite inadequate to supply the requisite vitamins if the demands of cattle were appreciable. That they are *not* is evidenced by our earlier data of 1915<sup>(11)</sup> in which adult cattle are recorded as fed upon polished rice with only 2 lb. of autoclaved straw as roughage. No



Photograph 5. Animal 804 for comparison with animals 925 and 866. In excellent condition on the same diet as animal 925, but supplemented with calcium and phosphorus. Although adequate in minerals the diet was deficient in vitamins A, B and C.



Photograph 6. Animal 882, in excellent health after 18 months on an acidogenic diet rich in phosphorus but low in calcium.

tremors were therefore felt in planning the present series of experiments, involving vitamine deficiency as incidental concomitant to mineral deficiencies; but at the same time it was thought advisable, in an experiment involving growth, and ultimately lactation, to include at least one vitamine "control." In this experiment *H*, therefore, the hay was cut down to 1 lb. and 4 lb. of young *green* forage (oats, barley, lucerne; whichever happened to be available) was added. This amount of green fodder, rich in all vitamins, but limited in order to minimise variation in other factors, was considered sufficient for purposes of "physiological contrast."

### RESULTS.

The experiment was commenced on September 15th, 1924, after a preparatory period to bring the animals gradually over from their previous grazing to the "low roughage high concentrate" ration. Open stalls with wooden barriers were used, and all the animals received at least a few hours' exercise each day in bare courts in *brilliant sunshine*. The "irradiation factor" is therefore ruled out. The courts were not paved, partly because paved courts were not available and partly because it was desired to observe the prospective development of hoof deformation under relatively natural conditions. The possible influence of "soil licking" must therefore at least be touched upon. It may at once be stated that this was rare and confined to animals in *Exps. A* and *C*. As will be evident from the results to follow, the limiting factor in *A* is phosphorus, so that possible supplementing of calcium by occasional licking of soil does not vitiate the experiment; while in *C* the excess of calcium in the diet is so large that "soil licking" does not matter. The theoretical possibility of vitiation of the experiments by using bare soil courts can therefore be *disregarded*.

The animals were weighed at fortnightly intervals, and tested for osteophagia once a week. The behaviour in this latter respect may be left out of present consideration since it has been dealt with in earlier work (*loc. cit.* (1), (8) and (9)). The records of weight and food consumption (Table II) may be summarised at quarterly intervals since intermediate variations do not bear specifically on the results to be discussed.

Surveying this table, it is apparent that the ration of *Exp. A*, supplying only 5.1 gm.  $P_2O_5$  and 6.9 gm. CaO per day, is deficient, but that animals from 12 to 18 months old withstand this degree of deficiency of both phosphorus and calcium fairly well for three to six months. Thereafter they showed definite signs of mineral deficiency disease. Presumably

Table II. *Abbreviated records of weight and food consumption.*

Experiment		Hay ration constant at 3½ lbs.: fanko increased <i>ad libitum</i> .																Comments at December 1926				
		Sept. 1924		Dec. 1924		March 1925		June 1925		Sept. 1925		Dec. 1925		March 1926		June 1926			Sept. 1926		Dec. 1926	
		Heifer No.	Wt. lb.	Fanko lb.	Wt. lb.	Fanko lb.	Wt. lb.	Fanko lb.	Wt. lb.	Fanko lb.	Wt. lb.	Fanko lb.	Wt. lb.	Fanko lb.	Wt. lb.	Fanko lb.	Wt. lb.		Fanko lb.	Wt. lb.	Fanko lb.	
A. Low Ca and very low P	925	460	5	540	6	640	7	650	7½	650	8	620	8½	—	—	—	—	—	—	—	Styfsiekte. Death February 1926. No calf	
	759	450	5	570	6	650	7	710	7½	770	8	820	8½	920	10	940	10	720	10	—	Still-born calf June 1926. Develop- ment of Styfsiekte then rapid. Death November	
B. Low Ca and low P	881	410	5	530	6	590	7	660	7½	730	9	760	9	800	9	820	9	920	9	700	9	Weanling calf in November 1926. Mother beginning to manifest signs of aphosphorosis in De- cember
	1020	370	5	420	6	530	7	620	7½	720	8½	820	8½	920	9	980	9	740	9	640	9	Healthy calf June 1926. Mother then developed Styfsiekte. Death in December
C. Adequate Ca and P	804	350	5	440	6	500	7	630	7½	750	9	880	9½	1020	11	1130	11	1150	12	1220	12	Healthy mother. Healthy calf in June 1926
	727	580	5	720	6	820	7½	920	7½	1020	10	1120	12	1280	14	1310	14	1130	14	1180	14	Calved in July 1926. Both still doing well
D. Low P but high Ca	922	520	5	550	6	610	7	660	7½	670	8	680	7½	720	7	—	—	—	—	—	—	Styfsiekte. Died April 1926 with mummified foetus <i>in utero</i>
	866	450	5	520	6	600	7	620	7½	630	8	650	8	720	7	800	7	550	7	—	—	Styfsiekte. Gave birth to an ab- normal calf in June 1926
E. Low Ca but high P	862	460	5	600	6	720	7½	810	7½	900	9	1000	10	1060	10½	1140	10½	920	10½	930	10½	Aborted twin calves in June 1926, but died of metritis following retention of after-birth
	923	480	5	630	6	760	7½	850	7½	940	9½	1030	10	1130	10½	1210	10½	—	—	—	—	Aborted an 8-months foetus in May. Mother healthy
F. Low Na and low Cl	872	470	5	560	6	670	7½	740	7½	830	10	930	11	1100	13	1230	13	1200	13	1240	13	Still-born calf in June. Mother healthy
	873	480	5	570	6	680	7½	770	7½	860	10	960	11	1120	13	1230	13	1270	13	1320	13	Waiting calf born in July. Mother died of metritis following retention of after-birth
G. Low Na, high K	875	470	5	550	6	660	7½	730	7½	730	8½	830	9	950	10	1000	10	—	—	—	—	Animal 878 dead. Animal 1590 healthy
	878	450	5	570	6	670	7½	720	7½	720	5	820	7	700	10	870	11	1030	12	1130	12	Calved normally in June. Both healthy
H. Vitamine supple- ment	869	470	5	570	6	660	7½	730	7½	830	10	900	10½	1020	11	1030	11	950	11	—	—	Calved normally in July. Both healthy
	927	510	5	630	6	720	7½	790	7½	920	10	1010	11	1190	12	1310	12	1060	12	—	—	



skeletal reserves are sufficient to allow of comparatively normal growth for several months, and it may be noted that both animals increase in weight by about 120 lb. during the first three months, or to practically the same extent as the animals in *Exp. C*, in which the phosphorus and calcium of the ration are nearly six times as high. During the following three months, however, the relative increase in *C* (180 lb.) is twice that of *A*. During the third quarter both animals in *Exp. A* showed unmistakable development of "Styfsiekte," much more marked in Heifer 925 than in Heifer 759.

*Styfsiekte*. Since the term "Styfsiekte" or "stiff-sickness" is a South African one, a cursory description of the disease is perhaps necessary. The naturally occurring economically important disease shows itself in retardation of growth and abnormal skeletal development, the most obvious features of which are the thickening of the epiphyses of the leg bones, most pronounced at the metacarpus and first phalangeal joints of the fore limbs. The head and horns are sometimes disproportionately long, and the whole skeleton is light. The hoofs grow out, and the points frequently curve inwards owing to separation of the digits. The gait is stiff and lame. In the young lactating animal there is usually rapid loss of condition, the lameness may become acute, and the animal tend to walk on its heels, with hoofs growing out. The bones may become fragile and fracture of the ribs and pelvis has been observed. The posture becomes abnormal, sometimes with curved spine, and in the last stages the stiffness and lameness become so painful that the animal is unable to rise, so that death from cachexia ensues. An early diagnostic sign, apart from stunting of growth, is the gradual separation of the claws of the hoof, and the appearance of a depression below the first phalangeal joint, deepening to a groove between the digits. The disease resembles that referred to in the European veterinary literature as "Osteomalacia" (8), and is probably identical with the "Cripples" of Australia.

In the case of Heifer 925, the first signs of *Styfsiekte* were distinct after six months on the phosphorus-low ration, and by June 1925, nine months after commencement of the experiment, were quite unmistakable. Thereafter the animal continued to grow without increasing in weight, showing increasing signs of general malnutrition, finally becoming so stiff and lame that it was unable to rise. It died of cachexia at the beginning of February 1926, after sixteen months on the deficient ration, and long before it was due to calve. Its companion, Heifer 759, showed the disease in less pronounced form, succeeded in doubling its weight in 18 months, but was nevertheless "stunted" in growth. After calving

in June, however, the drain of lactation, although the actual milk yield was small, rapidly accentuated the development of Styfsiekte and the animal was killed *in extremis* on November 23rd. For some time before death it was incapable of rising. The calf was still-born, and weighed only 59 lb. There is little doubt that the extreme aphosphorosis of the mother was responsible for the failure to produce a healthy calf, although the cow herself survived in poor condition for a further five months. Over this period of five months the milk yield ranged from 3 litres down to 1 litre per day, and even less in the last phases preceeding death. Even for an animal of this "veld cross-bred" type, which usually only yields sufficient milk for the minimum requirements of its calf, the actual yield recorded is exceptionally low; being at its highest point less than half of that yielded by animal 727 of *Exp. C*, on the same ration except for the bone-meal supplement. The percentage composition of the milk, however, was almost normal, the CaO ranging around 0.16 per cent. and the  $P_2O_5$  around 0.21 per cent. When it is considered that the initial output of 3 litres involved, through the *milk alone*, a daily loss of 6.3 gm.  $P_2O_5$ , or approximately as much as the total present in the food at the time, it is easy to understand the rapid accentuation of the disease after calving. On the assumption that the Styfsiekte symptoms prior to June 1926 are evidence of a negative phosphorus balance before calving, the small milk-yield alone represents a minimum disappearance of about an ounce of "fresh skeleton" daily. At the time of death the ribs were fragile and brittle, while the whole skeleton was light<sup>1</sup>.

Since these are the first recorded cases of experimentally produced aphosphorosis of cattle, characteristic photographs are worthy of reproduction. Photograph 1 shows Heifer 925 after 15 months on the phosphorus-deficient ration and illustrates the stiff gait to which the name "Styfsiekte" owes its origin. Photograph 2 illustrates the malformation of the legs of the same animal.

If the weights of Heifers 922 and 866 of *Exp. D* be next examined, it will be noted that the addition of chalk, raising the total CaO from 7 gm. to 29 gm. does not modify the adverse effect of low phosphorus but, if anything, intensifies it. Both showed Styfsiekte and poverty, with rough staring coat, and a weight about 200 lb. below that of Heifer 759 in March 1926. Animal 922 died on April 30th with a dead mummified foetus *in utero*. On June 18th its companion, animal 866, gave birth to an abnormal calf (61 lb.) showing elongated claws and

<sup>1</sup> The pathology and symptomatology of the disease are being dealt with by Theiler in a separate article. Chemical composition of the bones will appear in a later article by Green.

thickened joints, particularly of the knees. It might almost be described as showing Styfsiekte at birth, and was killed for pathological material. The mother survived a further three months, yielding from 5 litres down to 1 litre of milk per day. This additional drain of phosphorus rapidly accentuated the disease and the animal literally "went to pieces." It fractured several ribs in falling, and was put out of agony on November 7th. Photograph 3 illustrates the painful kneeling posture adopted by animal 866 at various times during the weeks preceding death. Photograph 4 shows the malformation of its calf.

These two cases in which typical Styfsiekte was produced on a diet high in calcium but low in phosphorus, combined with the fact that the naturally occurring disease can be prevented by supplementing the grazing of the areas in which it is prevalent, with phosphorus compounds only (*e.g.* bran, phosphorus high but calcium low) definitely established the Styfsiekte of South Africa as a *straight aphosphorosis*.

From the theoretical point of view it may be noted that in spite of what, in the sense of Marek<sup>(5)</sup> and Ibele, would be called a favourable "Erdalkali-Alkalizität," the absolute daily intake of phosphorus is so low that specific deficiency disease is produced.

Passing next to *Exp. C*, in which the intake of both calcium and phosphorus is raised by the addition of 100 gm. of bone meal, the difference is very striking. Both animals grew normally and maintained excellent condition right throughout the experiment. From Table II it will be noted that animal 804 trebles its weight in 18-months, steadily increasing from 350 lb. in September 1924 to 1020 lb. in March 1926, and to 1220 lb. in December 1926. Its companion, animal 727, reached approximately 1300 lb. in March, and although it suffered a rather greater set-back after calving in July, it was in excellent condition at 1200 lb. when discharged from experiment in December. Photograph 5 shows animal 804 taken about the same date as photograph 3. The only material difference between the diets of the two animals was the phosphorus and calcium content.

Turning now to the weight records of *Exp. B* in Table II, it is apparent that the addition of only 25 gm. of bone meal to the basal ration, bringing the total CaO to 15 gm. and  $P_2O_5$  to 11 gm., is not sufficient for growth, although actual deficiency disease did not become definite until after calving. Heifer 1020 does fairly well on this quantity of phosphorus for 18 months, increasing in weight from 370 lb. to 920 lb. and remaining in fair condition, although inferior to Heifer 804 which gained 100 lb. more over the same period. On June 26th it gave birth to a normal

healthy calf, but the drain of lactation resulted in rapid development of Styfsiekte, continuous decrease in condition, and death on December 17th (killed at acute stage). Its milk yield varied from 5 litres a day down to rather less than 3 litres shortly before death. Since 5 litres of its milk alone contained 11 gm.  $P_2O_5$ , or approximately the total in the food, the rapid development of aphosphorosis was inevitable.

Its companion, Heifer 881, behaved in much the same way, calved normally in November, but in spite of the higher milk yield of 6 to 7 litres per day, did not show clear clinical signs of deficiency disease until January; although, as will be shown presently, it clearly reflected aphosphorosis by a reduced "inorganic phosphorus fraction" of its blood, some months in advance of the appearance of clinical symptoms. Its calf was weak, however, and died five days after birth, with symptoms of enteritis.

*Exp. E* is of special interest since both animals do very well for the first 18 months of experiment, in spite of the low calcium intake. Up to this time they behave almost exactly like those in *Exp. C*, scaling approximately 1100 lb. in excellent condition in March 1926. The supplement of bran raises the total  $P_2O_5$  to 24 gm. but still leaves the total CaO as low as 8.2 gm. Up to the date of calving no sign of malnutrition appeared, and 8 gm. CaO per day, chiefly derived from the small hay moiety, is apparently sufficient for normal growth of this class of cattle. Both cattle, however, calved abnormally. Animal 923 gave birth with difficulty to a normal calf (84 lb.) on June 27th, but retained the after-birth, and in spite of skilled treatment died of gangrenous metritis and peritonitis shortly after. Its companion, animal 882, aborted twin calves on June 10th, was treated for retention of after-birth, suffered from chronic metritis for a considerable time, and lost heavily in condition. In how far these mishaps can be specifically related to the low calcium of the diet may be left an open question until further data are acquired. Up to the date of writing (January 1927), six months after the abortion, animal 882 has continued to yield an average of nearly 4 litres of milk per day, with a normal CaO content of 0.19 per cent. This represents a daily elimination in the milk alone of an amount of calcium almost as high as that present in the diet, and the fact that no clear symptoms of calcium deficiency disease have yet appeared points to very economical utilisation of calcium under conditions of low calcium intake.

For the first period of the experiment both of these bran-fed animals increased approximately half a kilogram per day for 18 months on the low absolute daily intake of 8.2 gm. CaO and relatively high intake of

24 gm.  $P_2O_5$ . Skeletal development was normal and on the usual method of reckoning CaO as stored to the extent of 1 per cent. of the live weight increase, 5 gm. CaO would be stored per day; or 60 per cent. of the total in the ration. The extent of absorption of the calcium in the small hay ration (the major source) must have been very perfect if so large a proportion can actually be stored in the skeleton. But, however perfect the absorption, there is no doubt that during the subsequent lactation period the surviving animal 882 has drawn upon its skeletal reserves for milk production, and the surprising feature is that no clinical evidence of calcium deficiency, beyond failure to recover the weight lost after abortion, has yet been manifested.

A further point of interest in this experiment *E* is that the moderately high ratio  $P_2O_5$  to CaO, of 3 to 1, with the phosphorus in the easily assimilable form of phytin (bran), seems to have increased rather than diminished the utilisation of the small proportion of calcium in the hay; a behaviour contrary to the conceptions of those investigators who maintain that excess of phosphorus over calcium is disadvantageous (*loc. cit.* Ingle<sup>(6)</sup> and Marek<sup>(5)</sup>). Indeed, ignoring the complications of lactation, for which the calcium in the ration is obviously too low, and considering only the records for growth, between September 1924 and June 1926, during which period the animals of *Exp. E* increased from an average weight of 470 lb. to an average of almost 1200 lb., the data can be profitably used to discuss the base-acid ratio upon which several German authors lay so much stress.

Expressed in terms of "milligram equivalents" (or c.c. normal reagent) the total base of the initial ration works out approximately at CaO, 293 (8.2 gm. divided by equivalent weight 28 and multiplied by 1000), plus MgO 545, plus  $K_2O$  660, plus  $Na_2O$  484; or a total of 1982 mg. equivalents. The total acid works out approximately at  $P_2O_5$  1013 (or 600 if all absorbed and reckoned as rather less than divalent for purposes of physiological regulation of neutrality), plus Cl 564, plus  $SO_3$  325; or in all 1902 mg. equivalents. The ration is therefore potentially slightly basic to begin with, but since the fanko is potentially acid the whole ration becomes slightly acidogenic in the later stages of the experiment; in reality definitely acidogenic from the beginning, owing to superior absorption of acid ions during digestion. That this empirical calculation, which considered alone would be of very doubtful significance, does roughly reflect the true physiological acid-base balance, is indicated by the fact that the *reaction of the urine* of these bran-fed animals was markedly acid; being, in the later stages of the experiment, pH 6.5

as against pH 7.5 for the animals in *Exp. D*. The fact that the reaction of the urine was actually on the acid side during the whole period of growth at a rate normal for the class of animal concerned, seems to us to dispose of the anxiety of many authors who insist upon a preponderance of base in the ration.

It is now easy to show the fallacy of Marek's (*loc. cit.* (5), p. 9) emphasis upon "Alkali-Alkalizität" and "Erdalkali-Alkalizität." The total preponderance of  $K_2O$  and  $Na_2O$  over  $Cl$  and  $SO_3$  works out at 255 mg. equivalents, which, divided by the *dry matter* of 3.7 kilos, gives an "Alkali-Alkalizität" of plus 6.9 in Marek's sense. By the time the fanko has increased to 10 lb. this figure falls to about plus 3. What then becomes of Ibele's contention (supported by Marek (5), p. 9) that in herbivora the "Alkali-Alkalizität" should be at least plus 17, and that if it is too low the disease "Lecksucht" develops?

In regard to "Erdalkali-Alkalizität" the fallacy is even more striking. The total "milligram equivalent" of the initial ration works out at  $CaO$  293, plus  $MgO$  545, minus  $P_2O_5$  1013; or *minus* 175 on a dry matter of 3.7 kilos. Per 100 gm. it is therefore minus 4.7, or quite definitely negative. According to Marek this figure should be at least plus 25, and a negative value leads to rachitis and osteomalacia. Photograph 6, of animal 882, after 18 months' feeding on this acid diet containing three times as much  $P_2O_5$  as  $CaO$ , is offered by way of contradiction of Marek's views. By way of further contrast photograph 3 of animal 866 may be again referred to, showing Styfsiekte (analogous to osteomalacia) on a diet in which the "Erdalkali-Alkalizität" is about plus 30 (*i.e.* correct in Marek's sense) but in which the absolute daily intake of phosphorus is too low.

In regard to this whole question of ratio of bases to acids, particularly of  $CaO$  to  $P_2O_5$ , it is dangerous to generalise in the present state of our knowledge. Indeed, might not the reverse standpoint to that adopted by Marek (and Ingle) be defended? When the  $CaO$  is low may a relatively high proportion of  $P_2O_5$  not actually facilitate absorption of calcium from the gut and so reduce the risk of calcium starvation? *Exp. E* suggests this. When  $P_2O_5$  is low may not a relatively high  $CaO$  reduce absorption of phosphorus from the gut and so increase the danger of aphosphorosis? *Exp. D* suggests this. Is it not likely that the base-acid ratio in dietaries only becomes important when the preponderance of one over the other becomes so great that the liberal physiological mechanisms for preservation of neutrality of the tissues are strained?

*Exps. F, G and H* may be more rapidly dismissed. In *F* the point of interest is the low sodium and low chlorine content. The total  $\text{Na}_2\text{O}$  is only 2 gm. per day, equivalent to 3.8 gm.  $\text{NaCl}$ . The total chlorine, however, is 5 gm., or more than twice the sodium equivalent. Potassium preponderates heavily,  $\text{K}_2\text{O}$  being 24 gm. or twelve times as high as  $\text{Na}_2\text{O}$ . The average weight of the two heifers concerned was 475 lb. in September 1924, 1110 lb. in March 1926, and 1280 lb. when discharged in December, in excellent condition. Neither, however, calved normally. Animal 872 aborted an eight month foetus on May 3rd, and from animal 873 a still-born calf weighing 78 lb. was born on June 24th. Both mothers remained healthy, and on milking yielded about 2 litres of milk of normal composition. Whether the calving mishaps have any relation to the low sodium intake must remain an open question until more extensive data are acquired, but in so far as growth is concerned no adverse effect could be detected. In view of the commonly accepted belief that a fairly high salt ration is required for cattle, this result is somewhat surprising. The whole question is now being studied under practical conditions on a farm in the Ermelo district on which the grazing has been found to vary from .004 per cent.  $\text{Na}_2\text{O}$  downwards. The same vegetation shows over 0.4 per cent.  $\text{K}_2\text{O}$ , or one hundred times as much potassium as sodium. Meanwhile it is of interest to note that the animals of *Exp. F* on 2 gm.  $\text{Na}_2\text{O}$  per day, grow out just as well as those of *Exp. C*, on the same ration except for the addition of salt, bringing the total  $\text{Na}_2\text{O}$  to approximately 16 gm.

In *Exp. G*, dealing with the ratio of  $\text{K}_2\text{O}$  to  $\text{Na}_2\text{O}$ , both animals thrived well from September 1924 to May 1925 on the ration containing 44 gm.  $\text{K}_2\text{O}$  but only 2 gm.  $\text{Na}_2\text{O}$ . On doubling the allowance of  $\text{KCl}$  in May, increasing the total  $\text{K}_2\text{O}$  to 64 gm. (ratio 32 to 1), Heifer 878 died within a few days with symptoms of tympanitis, but showed no characteristic post-mortem lesions upon which to base a specific diagnosis. Its companion, animal 875, became ill, lost appetite and declined steadily in weight for two months; lost 70 lb., then picked up in weight and proceeded in good health at its earlier rate of increase until it calved on July 3rd. The calf was small (59 lb.), born weak, and died five days later. The mother suffered from severe metritis, following retention of after-birth, did not respond to treatment, and was killed *in extremis* on July 29th.

Animal 878 was replaced by animal 1590, but the potassium chloride supplement maintained at 32 gm., leaving the ratio of  $\text{K}_2\text{O}$  to  $\text{Na}_2\text{O}$  at 22 to 1 (44 gm.  $\text{K}_2\text{O}$ , 2 gm.  $\text{Na}_2\text{O}$ ). It has not yet calved (January 1927),

but is in excellent fat condition, having increased in weight from 400 lb. to 1130 lb. in 15 months.

The data at present available are too fragmentary to justify any generalisation, although it may be pointed out that no disease has been manifested, such as the "Lecksucht" attributed to this cause by German writers (*vide* (9)); and that so far as growth is concerned, relatively high proportions of potassium to sodium appear to have little influence. In any case it is doubtful whether any problematical retardation of development, or complications in calving, if such are ultimately shown to be influenced by the relative proportions of sodium and potassium in the diet, should be attributed to *ratio*, or to any effect in depressing utilisation of other minerals, along the lines suggested by Orr (*loc. cit.* (2)). Indirect sodium deprivation through possible influence of high potassium excretion on the ability of the kidney to conserve a low absolute sodium intake, remains to be experimentally considered. Thus an animal may behave very differently on an absolute intake of 2 gm.  $\text{Na}_2\text{O}$  with 64 gm.  $\text{K}_2\text{O}$  (the figure for animals 875 and 878), and 1 gm.  $\text{Na}_2\text{O}$  with 32 gm.  $\text{K}_2\text{O}$ , or 4 gm.  $\text{Na}_2\text{O}$  with 128 gm.  $\text{K}_2\text{O}$ , although the ratio is the same in all three cases.

*Exp. H* is of interest only in substantiating the present contention, based on our earlier data of 1915 (*loc. cit.* (11)), that vitamins A, B, and C do not enter into serious consideration under any conceivable *natural* conditions of cattle rearing. Just as the white rat can do without the antiscorbutic vitamin so necessary for the guinea-pig, so do cattle seem to be able to dispense with an exogenous supply of vitamins B and C. While the present work was in progress, Jones, Eckles, and Palmer (12) have produced evidence to show that vitamin A is a necessary factor in the diet of young calves, but at the same time they show that even wheat straw is a good source of this vitamin for ruminants.

In the series of experiments here recorded the vitamin content of the basal ration is, for all practical purposes, limited to the small amounts present in the  $3\frac{1}{2}$  lb. of poor quality ripe hay. *Exp. H* is the same as *Exp. C*, except for substitution of part of the hay by 4 lb. of *fresh green forage* which greatly increases the content in all vitamins. The average weight of the two heifers concerned (numbers 869 and 927) was 490 lb. in September 1924, 1105 lb. in March 1926, and 1150 lb. in November 1926, some months after normal calving. The corresponding average for *Exp. C* (animals 804 and 727) was 465 lb. in September 1924, 1150 lb. in March 1926, and 1200 lb. in November 1926, also after normal calving. The apparent difference in favour of the vitamin-deficient ration is of



course fortuitous, and due to uneven character of the ordinary cross-bred cattle used. Photograph 5, of animal 804, betrays no suspicion of vitamine deficiency, and there is nothing in the behaviour of any of the four animals to suggest any superiority of the vitamine-rich over the vitamine-poor ration. Apparently the requirements of cattle for vitamins A, B and C, from the period of weaning onwards at least, are so low that they are covered by a few pounds of roughage of indifferent quality. Vitamine D is not considered in view of the exposure to sunlight of all the cattle. The vitamine content of the milk of these animals was not determined, but in so far as yield is concerned there was no noteworthy difference between *Exp. H* and *Exp. C*.

*Milk analyses.* A limited number of analyses of representative samples of milk from one animal in each experiment was undertaken<sup>1</sup>, but need not be tabulated at the present juncture, since no outstanding differences, explicable on a dietary basis, could be established with certainty. Forbes and co-workers<sup>(13)</sup> have shown that the mineral composition of milk is considerably influenced by the stage of lactation, and our own data, particularly in view of the fact that the animals used were not of a dairy type, are not sufficiently extensive to enable all factors influencing normal variation to be taken fully into account.

Variation in "total solids" and in "fat," were such as might be expected from the class of cross-bred veld animal concerned, the milk yield of which is always low and erratic. Total "ash" ranged from 0.72 to 0.99 per cent., but although the lowest figure happens to represent animal 866, when suffering from acute aphosphorosis a-week before death, it is not so low as to call for special discussion. The milk of animal 759 showed an ash content of 0.80 per cent. two months after calving, at a time when aphosphorosis was acute; but the milk of animal 727, healthy on a diet rich in both phosphorus and calcium, showed a similar figure of 0.87 per cent.

Variations in percentage of calcium and phosphorus were even smaller, the milk of animal 866 showing 0.20 per cent. CaO and 0.22 per cent.  $P_2O_5$  a week before death, as against 0.22 per cent. CaO and 0.24 per cent.  $P_2O_5$  for animal 727 of *Exp. C*, in excellent health. The milk of animal 759 suffering from Styfsiekte on a diet low in both calcium and phosphorus, showed 0.22 per cent.  $P_2O_5$  and 0.17 per cent. CaO. But the milk of animal 873 of *Exp. F*, on high calcium and high phosphorus, showed an equally low CaO value of 0.17 per cent. As a broad physiological

<sup>1</sup> Milk and blood analyses with the temporary assistance of Dr G. J. R. Krige and Miss I. Lonstein, to whom acknowledgments are hereby made.

generalisation, it may therefore be stated that deficiency of phosphorus, so acute as to lead to specific disease, is not necessarily reflected in the percentage composition of the milk, although it may become a limiting factor in milk yield.

Variations in chlorine content ranged from 0.11 to 0.15 per cent., but were arbitrary in the sense that the milk of animal 872 on the diet of lowest chlorine content (*Exp. F*) showed an intermediate figure of 0.13 per cent. Apparently the individualistic udder maintains the composition of its physiological secretion independent of very wide variations in the mineral components of the food, and even independent of wide variations in certain constituents of the blood. As will be noted below, the "inorganic phosphorus" fraction of the total phosphorus of the blood of cow 866 dropped to about a quarter of the normal value before death, at a time when the total phosphorus of the milk was normal. The "lipoid phosphorus" of the blood, however, remained normally high; an interesting corollary to the evidence provided by Meigs, Blatherwick and Cary<sup>(14)</sup>, that it is from this fraction of the blood partition that the phosphorus of the milk is derived.

*Blood analyses.* In the later stages of the experiments a sudden access of temporary assistance (Dr Krige) enabled a considerable number of detailed blood analyses to be executed, which point the path to wider investigations. A representative selection of these is offered in Table III,

Table III. *Analysis of whole blood.*

Inorganic elements in milligrams per 100 c.c. Haemoglobin by Newcomer Disc. Normal blood = 100.

Experiment ...	A			B				C	D	E	F	G	H	Calf 24 hours
Date 1926	Sept. 22nd	Nov. 23rd	At death	Oct. 10th	Nov. 23rd	Jan. 31st	Dec. 13th	Nov. 15th	Oct. 27th	Sept. 27th	Nov. 11th	Oct. 25th	Sept. 27th	after birth of
No. of animal	759	759	925	881	881	881	1020	804	866	882	873	1590	869	881
Total P ...	11.3	11.3	11.7	15.8	14.3	13.3	11.4	17.5	11.5	15.5	15.4	18.9	16.1	35.2
Inorganic P ...	2.5	1.3	1.3	2.8	1.3	1.3	1.4	5.8	1.5	5.1	4.0	6.2	5.1	8.2
Lipoid P ...	6.5	6.3	7.0	8.7	8.5	8.6	7.6	8.8	7.2	8.4	7.4	9.3	9.1	1.7
Organic acid- soluble P }	1.8	2.5	2.2	3.1	3.5	3.1	2.3	2.8	1.8	1.6	3.3	3.1	2.0	13.2
Ca ...	8.9	9.1	10.0	9.0	8.7	9.5	9.9	10.4	8.8	10.3	8.9	9.6	10.4	11.5
Mg ...	—	5.1	—	—	5.4	—	—	4.5	4.3	—	4.6	—	—	6.5
K ...	—	82	—	—	61	—	64	49	40	53	50	53	56	168
Na ...	—	276	—	—	293	—	273	279	303	—	301	300	—	241
Cl ...	—	285	—	—	280	—	290	285	200	—	280	305	—	315
Haemoglobin	86	80	90	—	111	109	98	115	92	92	115	98	112	95

in which data are expressed in mg. inorganic element per 100 c.c. of whole blood; whole blood because this is easier to handle through the post office in the event of the data proving to be of diagnostic value in field work. Haemoglobin, however, of value in correlating these data

when further laboratory studies include separate determination on plasma and corpuscles, is expressed on the Haldane standard of "normal human 100"; actually determined with a calibrated Newcomer disc.

It will at once be noted that the outstanding *characteristic of the blood of all the animals suffering from aphosphorosis is the low inorganic phosphorus*, and the correspondingly reduced total phosphorus. Taking the average of animals 804, 882, 873, 1590 and 869, all of which had sufficient food phosphorus for normal health, the figure 5.2 mg. inorganic P is obtained for normal whole blood. Excluding animal 873 would give a higher average for normal blood. Taking the average of the lowest figures reached by animals 759, 925, 881, 1020 and 866, all cases of "Styfsiekte," the figure 1.3 mg. is obtained; only one *quarter of the normal*. The fact that animal 759 drops from 2.5 mg. in September to 1.3 mg. on the day before death in November, and that a progressive drop from 3.5 mg. to 1.3 mg. over 4 months has been observed in another case of Styfsiekte not registered in Table III, suggests that low inorganic phosphorus is definitely *diagnostic of aphosphorosis*. Animal 881 on a phosphorus ration above that of animal 759 but below that of animal 804, is of special interest in that clinical signs of Styfsiekte were not definitely manifested until January 1927, two months after calving. Yet at the date of the first analysis on October 10th, the inorganic phosphorus of the blood was as low as 2.8 mg. per 100 c.c. or approximately half the normal figure; and two days after calving was down to 1.3 mg., the figure characteristic of the later stages of the disease. If subsequent work shows that it is the rule for the inorganic phosphorus of the blood to drop some months before manifestation of clinical symptoms upon which a diagnosis of Styfsiekte can be made, a most valuable method for detecting incipient aphosphorosis becomes available.

In regard to lipid phosphorus, it is noteworthy that no characteristic difference is discernible between the different bloods. The figures for animal 925 on the day before death, and for animal 866 suffering from acute Styfsiekte, are not materially different from those for healthy animals 804 and 873.

The figures for "organic acid soluble phosphorus" (*i.e.* the fraction carried through into a trichloroacetic filtrate along with the inorganic phosphorus) need not be considered, since they are small and variable in normal animals.

The figures for calcium on the whole blood run a little lower in the blood of the phosphorus-deficient cattle, but the differences lie within the range of variation of normal cattle and it would, therefore, be unwise

to attach any significance to them. Sodium and chlorine are comparatively constant throughout, and in any case within the limits of normal variation. Potassium is known to be a constituent liable to very considerable normal variations for which it is not always possible to assign a cause, and it is, therefore, of interest to note that the added KCl of the ration of animal 1590 has *not* raised the potassium level of the blood.

The figures for the blood of the *calf* of animal 881 (Styfsiekte) the day after birth are exceedingly interesting, but may be left over for consideration in connection with further work. The figure for lipid phosphorus is low but the high figures for total phosphorus and inorganic phosphorus are in line with the earlier data of Meigs, Blatherwick and Cary (14) on normal calf blood. It would, therefore, appear that, so far as the phosphorus partition is concerned, the foetal blood is comparatively normal although the maternal blood is abnormal. Nevertheless the calf was born a weakling and died, with symptoms of enteritis, five days after birth.

*Urinary calculi.* At post-mortem, animals 866, 759 and 1020 showed abundant kidney stones. These were collected for later study.

#### SUMMARY.

1. Records are offered of year old cattle reared to adult weight on rations of varying content in respect to calcium, phosphorus, sodium, potassium and chlorine. Minimal requirements for growth are higher in the case of phosphorus than in the case of calcium, and a ratio of  $P_2O_5$  to CaO so high as three to one is not necessarily disadvantageous. Sodium requirements for growth are very low, 2 gm.  $Na_2O$  being more than sufficient. Chlorine requirements are below 5 gm. per day. A relatively high ratio of potassium to sodium is not productive of specific disease. There is no good reason to suppose that excess of basic over acidic constituents is necessary in a dietary, and cattle can grow normally to full adult weight when the usual alkaline reaction of the urine is shifted to the acid side.

2. Explanation of certain observed abnormalities in calving is left open, except in the case of phosphorus deficiency, in which definitely abnormal calves may be born.

3. "Aphosphorosis," or clinically recognisable phosphorus deficiency disease, is experimentally produced, and shown to be identical with the naturally occurring South African disease Styfsiekte.

4. The chemical composition of the milk of animals suffering from aphosphorosis need not necessarily be abnormal, but the "inorganic phosphorus" fraction of the blood may drop to a quarter of the normal value even before the disease can be diagnosed clinically. Other phosphorus compounds of the blood remain normally high. Blood calcium remains practically normal.

5. Vitamine deficiency of the diets had no adverse effect. Exogenous requirements of cattle for vitamins A, B, and C are so low that they are covered by a few pounds of poor quality roughage, and therefore do not enter into consideration under any natural system of cattle rearing.

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# SOME RECENT RESEARCHES ON SOIL COLLOIDS.

## A REVIEW.

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ALTHOUGH soil colloids, both organic or inorganic are known to occur normally as gels, their properties have generally been investigated in the sol state. This is natural, because in the present stage of development of colloid chemistry the properties of sols can be expressed far more quantitatively than those of gels. The greatest restraint is therefore necessary in using the results of experiments with sols in order to describe the properties of the complex gel system in the soil. In this connection Wiegner<sup>(1)</sup> points out that the factors influencing the degree of dispersion are qualitatively the same for gels as for sols.

Two soil colloids have been studied, clay and humic matter, neither of which is a chemical individual. The clay (Ger. Kolloidton) usually represents all the inorganic particles of equivalent diameter less than  $2\ \mu$  (United States Department of Agriculture clay particles  $< 5\ \mu$ , ultra-clay particles  $< 1\ \mu$ ) and the humic matter, the organic colloid which can be extracted with alkali. As ordinarily prepared, however, each of these is contaminated to some extent with the other.

It should be noted also that in certain types of soil the silt and even the fine sand may play a considerable part in the adsorption and base exchange phenomena which are often ascribed wholly to the colloidal clay.

### CLAY.

*Composition.* The American workers<sup>(2, 5, 6, 14)</sup> employ the high speed centrifuge for the separation of the clay from the coarser particles and obtain a product whose particles have an equivalent diameter of  $< 1\ \mu$ . Bradfield<sup>(2)</sup> has subjected such clay to a further fractionation using the centrifuge and has obtained three fractions. The coarsest of these contained particles from  $1.2\ \mu$  to  $.4\ \mu$  in diameter. The two finer fractions, which Bradfield terms emulsoids *A* and *B* contain no particles visible by the ordinary microscope. Analysis showed little difference in composition between the finer emulsoid *A* and the coarsest fraction, but the alumina extracted by hydrochloric acid was less in the latter than in the former. The titration curves of the clay fractions from various soils

were similar in character and led to the conclusion that clay behaves as a true acid (3). Attempts to synthesise this acid by mixing sols of silicic acid, aluminium hydroxide and ferric hydroxide in the correct proportions gave a product whose properties were entirely different.

Joseph (4) has also attempted to fractionate clay by this method, but he found no true fractionation. The coarser fraction after being suspended in water and passed through the centrifuge at the same rate as before was only partially thrown out of solution. By repeated treatments almost the whole of the coarser fraction could be redispersed. He concluded that clay consists almost wholly of colloidal matter. He correlates many of the properties of clays with their  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio. Gile (5) and Robinson and Holmes (6) have also examined the chemical composition of the clay fraction using a much greater variety of soils than did Bradfield. They have shown that it may vary in composition between fairly wide limits. Three of its colloid properties, the heat of wetting, ammonia adsorbed and malachite green adsorbed all show a correlation with the ratio  $\text{SiO}_2/(\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3)$ . Attempts to fractionate the clay colloid by cataphoresis and dialysis as well as by chemical methods all failed.

It is of great interest to examine the colloid fractions of a number of soils known to be derived from well-defined rocks. In favourable cases it should be possible not only to identify the particles of microscopic size but also to compare certain of their surface properties—notably the electro-kinetic potential, base exchange and adsorption—with those of the true colloid. In this way we should learn something of the change from the well-defined mineral particles to the ill-defined aluminosilicate complex. Hendrick (7) in his studies of a Scottish drift soil has made a start with this work.

*Optical properties.* No comparative study has been made of the optical properties of clays from different sources. Nephelometric methods have been proposed for rapid determinations of the concentration of clay suspensions (8). Joseph (9) has shown that the addition of neutral flocculants did not affect the turbidity but alkalis markedly reduced the turbidity, the effect of the cations following the order



This order of cations suggests that the effect may be one of swelling, which would decrease the difference in refractive index between the particle and the dispersion medium. According to Rayleigh's formula (13) such a decrease would diminish the intensity of the Tyndall beam.

Wyckoff<sup>(5)</sup> has examined the X-ray spectra of several clays of differing  $\text{SiO}_2/(\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3)$  ratios by the powder method and found that each gave a distinct series of lines. Bachmann<sup>(10)</sup> noticed that kaolin suspensions showed the Zocher<sup>(11)</sup> effect on stirring and observing between crossed nicols. At suitable speeds of rotation a lightening of the field resembling whirling clouds occurs and a black cross radiating from the centre appears. In this laboratory the same effect has been noticed with clay from Rothamsted soil using particles of equivalent diameter less than  $0.5 \mu$ . At suitable concentrations and speeds of rotation the black cross effect is well marked. From the analogy between these properties and those observed in aged sols of vanadium pentoxide and ferric hydroxide<sup>(12)</sup> one may conclude that clay particles are non-spherical and anisotropic. Mineralogical considerations would lead one to expect plates rather than threads, but no experiments have yet been carried out to decide between the two alternatives.

*Adsorption.* Adsorption by the clay fraction of soil has mainly been studied with a view to developing methods for its estimation<sup>(14)</sup> or correlating it with the  $\text{SiO}_2/(\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3)$ <sup>(6)</sup> or  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios<sup>(4)</sup>. The adsorption of malachite green, ammonia and water vapour usually shows a rough correlation with one of these ratios. It is noteworthy that whereas the American workers<sup>(14, 15)</sup> ascribe practically the whole of the adsorption by soil to the clay fraction, Ogg and Hendrick<sup>(7)</sup> found that in a Scottish drift soil the fine silt fraction was of almost equal importance. Powdered minerals were compared with the soil fractions in both cases. Gile and his collaborators came to the conclusion that the adsorption of these was small compared with that of the soil. Ogg and Hendrick, using powdered granite, found an adsorption almost equal to that of the soil fractions of the same equivalent diameter. Gile (*loc. cit.*) lays great emphasis on the difficulty of removing aggregated colloidal matter from the coarser fractions. Most of the recent work, however, is concerned with three effects accompanying adsorption—namely, coagulation, electro-kinetic changes and base exchange.

*Coagulation.* (a) *Technique.* It is a feature of modern research on soil colloids that a large amount of work has been done on their coagulation by electrolytes. In this respect clay has been studied much more than humic matter. Most of the earlier work was carried out with suspensions of microscopic size and the results indicated that such suspensions behaved towards electrolytes as though they were negatively charged hydrophobe colloids.

Several methods are in use for the carrying out of quantitative experi-



ments on coagulation. They are based on the following facts(16). According to the amount of electrolyte added the sol may be brought into the region of slow coagulation or of rapid coagulation. In the region of slow coagulation the particles still retain a portion of their original charge, and the rate at which they coalesce shows a very sensitive variation with the amount of electrolyte present. The curve connecting the time taken to reach a given stage of coalescence with the amount of electrolyte is hyperbolic. Mattson(17) and Gallay(18) obtained curves of this type for clay. Increase in the amount of electrolyte brings us to the region of rapid coagulation; we are on that arm of the curve which runs parallel to the time axis. The rate of coagulation then becomes independent of the amount of electrolyte. It is usually assumed that in this region the particles are completely discharged but this may not always be the case(19).

Sven Odén(19) has shown that in the case of a clay the coagulation is much less simple than this owing to the particles varying in their sizes. The larger particles reach the region of slow coagulation at a lower electrolyte concentration than the smaller ones. Three critical concentrations can now be distinguished corresponding to (a) the point at which the larger primary particles are slowly coagulated, (b) that at which the secondary particles have united to form aggregates of approximately equal size ( $2-5\ \mu$ ) and (c) that at which the sol as a whole shows rapid coagulation.

The counting of the number of particles in a known volume of sol at definite intervals after addition of the electrolyte lies at the basis of the ultramicroscopic study of coagulation. This method is theoretically sound but tedious in application since it involves a large number of counts of the particles. Standard conditions of mixing must always be adhered to. The coagulating powers of different electrolytes can be compared by finding those concentrations which cause the same percentage decrease in the number of particles in a standard time. This method was used by Wiegner(20) and Gallay(18) in their comprehensive study of the effects of cations on clays (see below). A modification of it was used by Mattson(17) in determining the efficiency of humic matter and of colloidal clay as binding agents for quartz particles when precipitated by electrolytes. He found that the quartz when precipitated by calcium ions in presence of humic acid, clay, calcium phosphate or ferric hydroxide gave a coagulum which could not be peptised again by washing out the electrolyte with distilled water.

The more usual method is to make use of the change in optical

properties of the sol caused by coagulation. A specific degree of transparency is chosen and the time taken to attain it is found for the different concentrations of electrolytes. Using standard conditions of illumination such a method can be fairly accurate, but as usually carried out it is hardly more than semi-quantitative. A series of test-tubes is set up in a row with different concentrations of electrolyte. They are examined after a known time has elapsed and the state of coagulation of their contents is noted by eye. Many of the results discussed below have been obtained by this method, notably those of Gedroiz, Bradfield and Mattson. In view of the results of Joseph (*loc. cit.*) this method is in need of careful re-examination.

Gallay<sup>(18)</sup> has followed the coagulation of clay by using the Ostwald viscosimeter. He found that during coagulation the viscosity rose and that it reached a maximum when coagulation was complete. This divergence from the behaviour of true lyophobic sols, whose viscosity according to Einstein's equation should be independent of the size of the particles, was explained in two ways. It was due in part to the fact that the sols he was using were relatively concentrated, so that the particles influenced each other's motion, and in part to the hydration of the particles, which were not truly hydrophobic in character. The effect of the hydration was seen in the gradual decrease of viscosity with time after complete coagulation. The coagulum was shrinking, and giving up the water which it had enmeshed during coagulation.

The phenomena of partial coagulation and of precipitation in layers have been studied by Gallay<sup>(18)</sup> who has shown that they are due to wide variations in particle size in association with high concentrations of the sols employed.

(b) *The effects of ions.* In many respects clay sols resemble other well studied negative sols in their behaviour towards electrolytes. The coagulating power of the cations increases with their valency, the hydrogen ion is comparable with divalent cations in its effect and zones of coagulation are noticed with some trivalent cations. The clay is behaving as a typical acidoid<sup>(21)</sup>. Further, Gedroiz<sup>(22)</sup> and Wiegner<sup>(20)</sup> have shown that the flocculating power of the alkali cations increases in the series  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Rb}^+$ , and also in that of the divalent cations  $\text{Mg}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{Ba}^{++}$ . Wiegner showed that the effect was due to the differences in hydration of these ions, and that it was the same whether the clay was prepared in the usual way or whether it was saturated with any of these cations as exchangeable bases. Thus clay has some of the properties of hydrophilic colloids, for which such series of ions are

characteristic. Bradfield(3) has shown the need of hydrogen ion control in coagulation experiments with clay. He found that different acids coagulated clay at a  $pH$  of 3.8. The amount of potassium chloride needed to flocculate increased gradually with the  $pH$  up to the neutral point, when it rose rapidly to a constant value.

The influence of base exchange on the colloidal properties of clays has been studied by Gedroiz(10) and independently by Gallay(18) and Wiegner(20). Gedroiz saturated a clay soil with various mono- and bivalent cations, and then subjected the product to mechanical analysis. He found great variations in the amount of clay fraction according to the cation used. The monovalent cations, including hydrogen, which had only a small effect, increased the dispersion of the clay fraction in the order  $H^+$ ,  $K^+$ ,  $NH_4^+$ ,  $Na^+$ ,  $Li^+$ . The chlorides only were tried. In the case of sodium the effect was so large that 80 per cent. of the particles originally from 1 to 3  $\mu$  in equivalent diameter appeared to be reduced to 0.2  $\mu$ . These values, however, were deduced from the rate of settling, assuming that a decrease in the rate of settling corresponds to a decrease in the size of particles. This is strictly true only if the density of the particles is unaltered. Imbibition of water consequent on the conversion of calcium clay to sodium clay, could, however, result in a diminution of average density and an increase in effective radius of the particles. It is thus possible, as already pointed out(50), that Gedroiz's results were due, at least in part, to imbibition effects, and not wholly to a subdivision of particles. Amongst the cations of higher valency only magnesium had a dispersive effect. Calcium, iron, aluminium or barium diminished the dispersion of the clay fraction.

Wiegner and Gallay studied the coagulation of such clays with different replaceable cations, both by the ultramicroscopic and viscosimetric method. The sodium, potassium and ammonium clays were approximately equally sensitive to electrolytes. The calcium clay was much more sensitive, being coagulated by  $N/100$  calcium chloride while the potassium clay required  $N/10$ .

In all cases the bivalent cations were better precipitants than univalent, but the difference between the two became less as the concentration of the suspensions was increased. Using a potassium clay it was found that the effectiveness of the alkali cations increased from  $Li^+$  to  $Cs^+$ . The viscosity of the coagulum also increases in this order, which leads the authors to the conclusion that the most highly hydrated cations produce the smaller aggregates (Wiegner) or the least hydrated coagulum (Gallay). This seems rather a surprising result since the viscosity of the

original clays increases in the series K,  $\text{NH}_4$ , Na, just as one would expect from the results of Gedroiz. The confusion arises from the fact that the effect of hydration is difficult to separate from possible changes in particle size. The sols in which it is possible to study the influence of particle size alone on viscosity are just those which are not stable in concentrated suspensions. As noted on p. 319, the particle size only influences the viscosity when the particles influence each other's motion.

Joseph<sup>(24)</sup> maintains that the effects of neutral salts on clay can well be explained on a purely chemical basis. He found that the acidity developed on treating various clays with sodium chloride solution increased as the ratio  $\text{SiO}_2/\text{Al}_2\text{O}_3$  increased, reaching a maximum for pure silica. Different salts bring different amounts of silica into solution, the order of the cations being  $\text{Na}^+ > \text{K}^+ > \text{Ba}^{++} > \text{Ca}^{++}$ . This is regarded as a solubility effect, the solubility of the silicates or complexes being supposed to decrease in this order. He finds, however, that clay can adsorb acids to a considerable extent and the acid adsorbed can be removed by dialysis<sup>(25)</sup>.

Mukherjee<sup>(26)</sup> using purified silica and Ghosh<sup>(27)</sup> using hydrated manganese dioxide have concluded from their experiments that the adsorption theory alone can account for all the facts. Unfortunately the facts themselves are not free from doubt, as Kappen and Breidenfeld<sup>(28)</sup> have failed to observe acidity on treating purified silica with potassium chloride, and from other experiments on synthetic aluminosilicates conclude that the ratio of alumina to other bases such as sodium is the one which controls exchange reactions. The decomposition of neutral salts by colloids will be considered in greater detail in the section on Humic Matter.

Fodor and Schoenfeld<sup>(49)</sup> explain the whole of the colloid phenomena of clay on an adsorption basis. Their results are in some respects peculiar. They found that after treatment of their clay with alkali hydroxides the whole of the latter could be recovered by simple washing. Also using mixtures of chlorides they found that the latter always had a stabilising effect. Their paper, however, gives no details as to the composition, origin, or particle size of the materials employed, and so loses much of its value.

The effect of the anions in coagulation has been studied in detail only in the case of the calcium salts. It was shown by Hall and Morison<sup>(29)</sup> that for a kaolin suspension there were appreciable differences in the effects of different calcium compounds. The hydroxide has a much lower flocculating power than the other salts. This is usually ascribed to the

peptising tendency of the hydroxyl ion. The calcium salts have been studied in detail by Gedroiz<sup>(22)</sup>, Comber<sup>(30)</sup> and very exhaustively by Mattson<sup>(17)</sup>. Gedroiz showed that for a true clay the flocculating power of calcium hydroxide was superior to that of the sulphate, nitrate and bicarbonate, and equal to that of the chloride. Comber's experiments verify this conclusion, and show also that the order is completely reversed in the case of the silt fraction, which also falls into line with the experiments of Hall and Morison, since they worked with a comparatively coarse suspension of kaolin. Comber showed that in the precipitation of clay the effect of adding  $\text{CO}_2$  after calcium hydroxide was the same as if calcium bicarbonate had been used originally. This was not true of silt. He found also that a suspension of silica gel showed the same behaviour as clay while silicic acid sol resembled silt. He regards all small soil particles as consisting of two parts, a hard mineral kernel and a gel-like coating. The differences between silt and sand are due to the different proportions of these, the relative amount of gel increasing as the particles diminish in size.

The subject of coagulation by mixtures of electrolytes is one which has only just been attacked by the pure colloid chemists and they regard the interpretation of results of such experiments as very difficult<sup>(31)</sup>. The results of Gedroiz, Comber, Bradfield and Mattson, obtained with mixtures of salts and alkali hydroxides have not been generally recognised. Gedroiz, Comber and Mattson all found that the addition of small quantities of alkali hydroxides to calcium salts increased their power of precipitation. Gedroiz showed that this was also true for mixtures of sodium chloride and sodium hydroxide within certain limits. Mattson studied the whole problem of precipitation by calcium salts both in presence and in absence of alkali, using cataphoresis and adsorption measurements as well as coagulation experiments. He showed conclusively that in the case of clay very small concentration of calcium hydroxide showed a stabilising effect, as did also very low concentrations of alkali in presence of calcium salts. Comber<sup>(30)</sup> also showed that at concentrations below  $N/1400$   $\text{CaCl}_2$  was a better precipitant than  $\text{Ca(OH)}_2$ . Mattson showed that quartz and humic matter did not show this behaviour, but only the increased coagulating power due to hydroxyl which is shown by clay in higher concentrations. Adsorption experiments showed that adsorption of calcium ion was much increased by the alkali. The coagulation in presence of hydroxyl took place while the particles still carried a much higher charge than was found for the coagulum produced by simple salts. The action of caustic soda on clay

particles was to increase the charge to double that in pure water at a concentration of  $\cdot 0002 N$  and at higher concentrations to decrease it. Calcium sulphate on the other hand always decreased the charge. When caustic soda was added in increasing concentration to calcium sulphate the charge was increased, even at concentrations of caustic soda greater than  $\cdot 0002 N$ . Increase in the calcium sulphate always decreased the charge. Mattson explained these facts by assuming that there was a preferential adsorption of hydroxyl ions which tended to raise the charge. Opposed to this tendency was the discharging effect of the cations. In the case of polyvalent cations, however, since each cation could be attached to more than one hydroxyl the particles could be held together by the polyvalent cations. This would occur when the number of polyvalent cations was not nearly sufficient to cause coagulation by the usual discharging effect. This theory undoubtedly adequately explains the facts investigated by the author but further work is needed before it can be regarded as definitely established. One fact discovered by Gedroiz seems to tell against it (22). He found that sodium hydroxide and sodium chloride showed a similar effect with regard to clay, namely that in low concentrations of salt, addition of caustic soda reduced the precipitating power, whilst at higher concentrations an enhanced power of coagulation was observed. On Mattson's theory we should not expect this to happen with univalent cations, unless we are to assume that the auxiliary valencies are the cause of the binding, and not merely the primary valencies. This type of phenomenon needs to be studied in many other colloid systems before a general theory can be stated. It is to be noted that the experiments of Mattson on the sensitivity of quartz suspensions to  $Ca^{++}$  in presence of  $OH^{-}$  do not agree with the theory of Comber. Comber (30) supposes that this "anomalous flocculation" is a property of the gel coating of the soil particles and that the mineral core does not possess it. It is on this distinction that his whole theory rests. The suspensions used by Mattson consisted of particles  $\cdot 5-1\cdot 4 \mu$  in diameter, which had been ignited before use, so that he was dealing with a true suspensoid. The experiments in which he proved the increased adsorption of calcium in presence of hydroxyl were carried out with these suspensions as well as many of the cataphoresis measurements which go to support his "link" theory.

Some discussion has taken place in *Nature* (May 1st, June 12th and Nov. 6th, 1926) between Joseph and Oakley and Kermack and Williams on "anomalous flocculation." Joseph and Oakley found, as Gedroiz had done previously, that sodium salts could cause the enhanced sensitivity

due to hydroxyl and wished on these grounds to abolish the term. Kermack and Williams attempted to show that it was the property of a silica coating round the clay particles, which showed the effect with calcium salts alone. Oakley showed that for purified silica the hydroxides both of Ca and Na were better precipitants than the chlorides and so proposed to restrict the term to the coagulation of silica and not to use it generally for differences between calcium and sodium hydroxides. With so many phenomena of this kind still unexplained it would probably be best to avoid the term altogether. With regard to the adsorption of hydroxyl in preference to most other anions this is an assumption which has been made widely in all branches of colloid chemistry. Miller<sup>(32)</sup> has shown, however, that in one system in which it has been assumed, namely charcoal, there is actually a negative adsorption of strong alkalis. Miller's charcoal differs from that of most other workers in being ash-free and rendered active by high temperature treatment. Miller has shown further that adsorption of neutral salts is hydrolytic. He has compared these results with the experience of those who have studied adsorption of soil, and finds a close parallelism. It may therefore prove that the preferential adsorption of hydroxyl is an assumption which is unnecessary. The electro-kinetic properties of this charcoal will have to be studied in great detail before the position is made clear.

*The electrical double layer.* The study of the electrical double layer by means of electro-kinetic phenomena<sup>(33)</sup> is becoming of great importance in the interpretation of colloid behaviour. The importance of the double layer in controlling coagulation has already been mentioned. In its essence, the measurement of the potential of the double layer depends upon the determination of the speed of two phases relative to each other under a known potential gradient. The measurement is usually carried out either by observing the movement of small particles relative to the liquid or that of the liquid relative to a fixed diaphragm, the former being termed cataphoresis and the latter electric endosmosis. In such measurements an accuracy of 1 per cent. has hardly ever been attained. So far measurements on soil colloids have been made only by the method of cataphoresis. This implies that their accuracy does not exceed 5 per cent., for no method has yet been proposed which has a greater accuracy than this. Attempts are being made to devise a more accurate apparatus in this laboratory. When a coarse powder is being investigated the method of endosmosis devised by Fairbrother and Mastin<sup>(34)</sup> affords a more accurate method of measuring the potential of the double layer than any other. With a uniform powder an accuracy of 1 per cent. can be attained.

Mattson<sup>(17)</sup>, who measured the velocity of cataphoresis of clay, humic acid and a quartz suspension, used a microscopic method. His results are comparative and only accurate at best to 5 per cent. Owing to the fact that his cell containing the liquid was not of uniform cross section it is not possible to calculate the potential of the double layer accurately. The results he obtained have already been discussed.

Bradfield<sup>(2)</sup> using Burton's U-tube method measured the velocity of cataphoresis of his clay acid, silicic acid, ferric hydroxide, aluminium hydroxide and a mixture of these last three in the same proportions as were found in the first. His results for the potential of the double layer were as follows (accuracy 5 per cent.): clay acid - 0.031 volt, silicic acid - 0.030 volt, ferric hydroxide + 0.030 volt, aluminium hydroxide + 0.067 volt, synthetic mixture + 0.041 volt. This showed that the natural clay acid was not identical with Bradfield's synthetic product. It is quite possible, however, that if he had used a variety of methods of preparation for his sols he might have obtained different results. He found that the effect of change in pH on the double layer potential was the same for the synthetic product as for aluminium hydroxide. In small concentrations acid raised the + charge.

Dayhuff and Hoagland<sup>(35)</sup> have done some cataphoresis experiments on clay colloid. Their method was similar to Burton's U-tube method and the accuracy attained was probably not greater than 10 per cent. They used a gelatinous lake-side clay as starting material. They found that the cataphoresis was constant between pH 5 and 10, a most surprising result. Some qualitative coagulation experiments seemed to show that sodium hydroxide was a better precipitant than sodium chloride, and it also lowered the cataphoretic velocity more. Unfortunately no analyses of the clay are given and no indications of its geological origin. It always carried a negative charge and after purification by washing had a pH of 8.5. It was evidently not identical with the material used by Bradfield. One cannot draw any general conclusions from these results.

The relation between electro-kinetic effects and base exchange has recently been examined by Mattson. He found<sup>(36)</sup> that electro-dialysis was capable of removing quantitatively the exchangeable bases from clay. He used two clays, one with a high  $\text{SiO}_2/(\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3)$  ratio and high adsorption and the other with a low ratio and low adsorption. In both cases the amounts of uni- or bivalent bases removed were the same as those removed by extractions with ammonium chloride or dilute hydrochloric acid. Further, in the electro-dialysed clay the exchangeable



hydrogen corresponded exactly to the bases removed; it could be almost qualitatively replaced by calcium, and this quantitatively removed by further electro-dialysis. Knowing the number and size of particles in his clay, Mattson was able to calculate that the number of univalent ions on each particle is about  $5 \times 10^5$ . On the other hand, by using his own value for the double layer potential and Stock's approximate value for its thickness (determined actually for quartz in nitrobenzene) he finds that the number of unit charges per particle is about 500-1000. Thus the double layer is not the only seat of exchange reactions, and a large part of the exchangeable bases exist elsewhere. Wiegner<sup>(46)</sup> using different figures has arrived at the same conclusion.

In another important paper<sup>(37)</sup> Mattson shows that methylene blue can reverse the charge of clays and give a well-defined isoelectric point at which adsorption of dye is complete. At this point the amount of methylene blue adsorbed was exactly equivalent to the exchangeable base content of the clay so long as the pH was the same in both determinations. Clays previously saturated with Na and Ca respectively gave the same isoelectric point with methylene blue, and at this point these ions were quantitatively displaced by it. These results were true for clays of widely differing  $\text{SiO}_2/(\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3)$  ratios, and it was noticed that there was a parallelism between this ratio and the content of exchangeable bases.

Oakley<sup>(38)</sup> in a theoretical paper has attempted to explain the electrokinetic properties of clay in terms of the Donnan membrane equilibrium. Since, however, his hypothesis is incapable of explaining the rise in double layer potential which occurs when the sol is treated with small amounts of alkali hydroxides or of alkali chlorides (though it does predict a rise when both are present together) it cannot be considered as final.

#### HUMIC MATTER.

From the colloid-chemical point of view the study of the organic material of the soil has not proceeded very far. The work done up to the year 1919 is fully discussed by Sven Odén<sup>(39)</sup> and his nomenclature will be adopted here. Humic acid is the alcohol-insoluble and hymatomelanic acid the alcohol-soluble portion of the organic matter of soils and peats which is extractable with alkali and precipitated by acids; humin is present in the alkali-insoluble portion. Odén has shown that humic acid behaves as a fairly weak tri- or tetrabasic acid with an equivalent weight of about 350. Hymatomelanic acid has an equivalent of about 200. It has been very little investigated. Most of the colloid work has been done

either on soil rich in humic matter (*e.g.* peat soils) or on a mixture of hymatomelanic and humic acids, extracted from the soil or peat by alkali and subjected to some purification processes. This mixture will be referred to as crude humic acid. Of the various artificial humic acids which can be prepared from phenols, furfural, sugars, etc., that prepared by oxidising hydroquinone with persulphate (Eller<sup>(47)</sup>) has been most used in the colloid work.

*Adsorption.* Kamamura<sup>(40)</sup> has studied the effects of acids and bases on artificial humic acid from cane sugar. He found that for acids the adsorption isotherm was followed in all cases but that the adsorption of phosphoric acid was greater than that of sulphuric, hydrochloric or nitric. The action of caustic soda or baryta corresponded with the neutralisation curve of a true acid except that beyond the neutral point both bases were appreciably adsorbed by the corresponding humates.

*Surface tension.* Ostwald and Steiner<sup>(43)</sup> have shown that the foaming capacity of humic acid from peat is not an inverse function of the surface tension. Humic acid in alkaline solutions shows no structural viscosity whilst in acid solutions the sols show a very marked effect. Ostwald has examined the ten Bosch dehydration process for peat from the colloid chemical point of view<sup>(42)</sup>. A simultaneous coagulation and peptisation is a feature of the process.

*Protective action.* Sven Odén<sup>(49)</sup> found the same protective action on clay whether neutral humate or the free acid in the sol form was employed. The protective action was strong, being of the same order as that of the proteins. Mattson<sup>(17)</sup>, however, points out that the action consists not only in a protection of the clay by the humus but also in a sensitisation of the humus by the clay. Mixtures of humic acid and clay are coagulated at concentrations of electrolytes intermediate between those necessary for each separately.

*The effects of ions.* A few rough determinations of the critical concentrations of five electrolytes for coagulation using the test-tube method were made by Odén<sup>(39)</sup>. Calcium humate was considerably less sensitive to electrolytes than humic acid.

Mattson<sup>(17)</sup> compared the behaviour of crude humic acid (from peat) with that of colloidal clay and a silica suspension, by the methods already referred to. He showed that for humic acid addition of alkali enhanced the sensitivity towards calcium salts, until the concentration of alkali became equivalent to that of the calcium, when the hydroxyl began to have a peptising action. This latter effect is supposed to be due to formation of sodium humate.

Ostwald and Steiner(43) have carried out extensive measurements of the coagulation of crude humic acid, using a preparation which was carefully freed from electrolytes. They used the viscosimetric method, a rise in viscosity indicating coagulation and a decrease peptisation. They paid particular attention to the changes in viscosity with time, which make the results not easy to interpret. The results show that the sol is less sensitive to electrolytes than sols of molybdic acid and vanadium pentoxide and that the effect of the valency of the cation is less marked. Artificial humic acid made from hydroquinone by the method of Eller and Koch showed very similar behaviour. The effect of electrolytes on the speed of sedimentation was also studied. Similar experiments, and in addition comparative measurements of the speed of filtration of electrolytes were carried out, using natural peat. It was found that chlorine water was a particularly good precipitant for peat suspensions. The best precipitants caused the greatest contraction of volume on drying.

Nolte(44) has investigated the effect of various cations on the permeability of a sandy soil rich in humic matter. The salt solutions used were much more concentrated than those employed in coagulation experiments. It was found that amongst the alkali metals the permeability increased with the atomic weight of the cation. On washing out the salt with water the soil became less permeable than before, the decrease in permeability being greater the lower the atomic weight of the cation. The alkaline earth halides showed a larger increase in permeability than the alkali halides, and after washing out there was less decrease in permeability. Trivalent cations caused a permanent increase in permeability. Also the permeability increased with the hydrogen ion concentration. These results if interpreted with regard to effect of the hydration of the cations are quite comparable with those of Gedroiz and of Wiegner on clays. The increase in permeability is due to a shrinkage of the gel, analogous to the coagulation of the sol. The subsequent decrease on washing is due to the greater hydration of the surface metal humate.

Gedroiz in his paper on "The Ultramechanical Composition of Soils"(23) examined the effect of salts on the dispersion of the humic portion of the soil. He showed that it is made more soluble by treatment with salts of lithium, sodium, ammonium and potassium. Subsequent treatment with water gives coloured filtrates which contain also peptised clay material. The hydrogen ion causes a slight increase in the dispersion of the humic material, while divalent and trivalent cations decrease it.

These results are quite analogous to those he obtained with the clay fraction.

The part played by humic matter in the phenomena of base exchange and neutral salt decomposition has been closely studied by Heimann<sup>(45)</sup>. He not only examined acid and neutral peat soils but also purified humic acids made from peats, lignite, sugar and hydroquinone. It had previously been shown by Kappen that whereas an acid mineral soil on treatment with a neutral salt gave up aluminium and ferric iron in amounts exactly equivalent to the acid liberated, this was not the case with an acid peat soil. In the latter case the acidity was greater than the equivalent of sesquioxides liberated. Heimann showed that humic acid itself can produce the apparent splitting of a neutral salt solution, in that it holds some of the base and sets free some of the acid. Salts with the same cation and different anions may show this effect to a different degree. If the humic acid is treated with an aluminium or iron salt, and then with a neutral salt it shows the same behaviour as an acid mineral soil. If treated with a neutral salt until no more acidity is produced it then behaves as a neutral mineral soil and exchanges bases in equivalent amounts.

The results of Heimann have been substantiated and extended by the work of Hümmelchen and Kappen<sup>(46)</sup>. They used humic acid from sugar and hydrated manganese dioxide which showed the neutral salt decomposition very plainly. Cupric hydroxide only gave a very slight effect while silicic acid and ferric hydroxide showed none. Equilibrium was established in a short time and was truly reversible. The relation between the concentration of salt and the acidity followed approximately the course of an adsorption isotherm, and the effects of alteration in volume of the solution, of the amounts of adsorbent and of the temperature, were in accordance with the usual adsorption experience. This was also the case with the effect of dilution using fixed amounts of adsorbent and salt. The acidity increased with dilution. Hümmelchen and Kappen regard this effect as abnormal but a little consideration will show that it follows from the adsorption isotherm. Consider the effect of doubling the concentration of salt. The acidity is increased in a smaller proportion than this, that is, the acidity per gramme of salt decreases as the concentration increases. Thus with a fixed amount of salt the acidity will increase with dilution, which is their experimental result. They found that in this neutral salt effect the order of the cations was



both for humic acid and manganese dioxide. The influence of the anions

in the case of manganese dioxide was in the order  $\text{Cl}' > \text{NO}_3' > \text{SO}_4''$  but for humic acids it became  $\text{SO}_4'' > \text{Cl}' > \text{NO}_3'$ .

The adsorption of free bases was found to be complete and irreversible at low concentrations, but incomplete and partially reversible at higher ones. It was made more complete by the addition of neutral salts, which agrees with the results of Mattson. The reversible adsorption of strong acids was not affected by neutral salts.

Manganese dioxide and silicic acid have been investigated from this point of view by Mukherjee(26). He has shown that both can cause neutral salt decomposition. He ascribes this to the preferential adsorption of anions, which is illustrated by the increase in negative charge of these substances when treated with neutral salts in low concentrations. The increase in negative charge for the different anions follows the order  $\text{C}_3\text{O}_4' > \text{SO}_4'' > \text{Cl}' > \text{Br}' > \text{NO}_3'$ , and the order for the decrease in charge due to the cations is  $\text{H}' > \text{Ba}'' > \text{Rb}' > \text{K} > \text{Na}' > \text{Li}$ . These series for silica agree well with those of Hümmelchen and Kappen for humic acid if one correlates an increase in the neutral salt decomposition with an increase in negative charge. Whilst Kappen explains his results by assuming a preferential adsorption of hydroxyl, Mukherjee ascribes the effect to the general preferential adsorption of anions which is characteristic (in his view) of negative colloids. It is evident that there is here much scope for further investigation.

In summing up the results so far obtained one may say that clay and humic matter each bears in its colloid properties the stamp of its origin. Clay is undoubtedly in the main hydrophobe in its colloid properties, and that in spite of the comparisons which are so frequently made between it and the sol of silicic acid. In its sensitivity to electrolytes it comes between the sulphur sol and the gold sol. The position of humic acid is more difficult to find. Its protective action places it amongst the truly hydrophile colloids, while its sensitivity to electrolytes is about the same as that of Odén's sulphur sols.

Insufficient material is available for the comparison of clay and humic acid gels with other gel systems of more definite composition.

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# THE MECHANISM OF CELLULOSE DIGESTION IN THE RUMINANT ORGANISM.

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DURING the carrying out of the series of researches which led to the formulation of the well-known expression for calculating the starch equivalent of a feeding stuff, Kellner<sup>(1)</sup> was led to investigate the value of crude fibre in the fattening ration of oxen. For this purpose he used material which had resulted from the boiling of rye straw with an alkaline solution under pressure, the object of this treatment being to free the cellulose of the straw from incrusting substances. This fibre-rich preparation was added to a basal ration which was slightly in excess of maintenance requirements. The result produced by the addition to the basal ration of the digestible matter derived from the fibre of the treated straw was found by Kellner to be equal to that produced by the addition of an equal weight of pure starch. The conclusion was therefore warranted that the digestion products of cellulose in the ruminant organism are equal, for purposes of fat formation in the body, to those derived from the digestion of starch. This finding is given practical expression in Kellner's formula for calculating the starch value of a feeding stuff, an equal value being attached to digestible fibre and digestible carbohydrate.

It is clear that any theory which is put forward to explain the breakdown of cellulose in the ruminant tract must be compatible with the experimentally demonstrated fact that the products of such digestion of a given weight of digestible fibre are equal in nutritive value to the products derived from the digestion of the same weight of starch.

The digestion of a carbohydrate like starch is brought about by the activity of enzymes, the starch being first hydrolysed to maltose and ultimately to dextrose. In addition to changes produced by enzyme action, however, carbohydrates are also subject to attack by bacteria in the rumen of the animal. As a consequence of such activity, a proportion of the carbohydrate of the food is fermented to gaseous products, like methane, and to simple fatty acids like acetic and butyric acids. The amount so destructively acted on is, on an average, about 8 per cent. of the total digestible carbohydrate of the food.



The hydrolytic breakdown of the stable cellulose molecule by means of enzymes has not been elucidated with the same degree of thoroughness as has the corresponding problem with starch. Such enzymes, however, known as cytases, have been shown to be present in seeds (barley, for instance). It has been demonstrated(2) that the breaking down of the cell wall of the endosperm cells, whereby nutrient matter is released for the nutrition of the embryo in the earliest stages of growth, is brought about by the action of a cellulose-dissolving enzyme which, though not present in the resting seed, is formed during the process of germination. Extracts of this enzyme are able to effect with rapidity the disintegration of the parenchymatous tissue of the potato, carrot, turnip, etc. A cytohydrolytic enzyme of great activity has also been shown to be present in the intestinal secretion of the snail(3).

Up to the present, however, investigation has failed to reveal the presence of cellulose-dissolving enzymes in the digestive tracts of the higher animals. It is safe to assert that the breakdown of cellulose in the ruminant tract is not the work of a cytase present in any of the digestive secretions. On the other hand, however, the possibility of the presence of such enzymes in certain types of plant food (*e.g.* cereal grains) should be borne in mind. Under the favourable conditions provided by the alimentary canal, cytases might become active in such plant food and thus cause the hydrolytic breakdown of cellulose. The writer(4) has shown, for instance, that the starch of maize, fed in the dry condition to pigs, is only slightly less digestible than the starch of cooked maize, and from this has drawn the conclusion that the cellulose coating of the starch cell is easily dissolved away during digestion. It is by no means improbable that the removal of the cellulose coating is effected by a cytase in the grain which becomes active under the conditions of the digestive tract. This possibility is strengthened by the fact that the trials were carried out on non-ruminant animals.

In the higher animals, however, it is now the accepted belief that the digestion of cellulose is mainly brought about by bacteria, which, in the ruminant, have their chief seat of activity in the rumen of the animal. The characteristics of such cellulose-splitting bacteria have been studied by numerous observers. Omelianski(5), working in artificial media seeded with cultures of the bacteria, has demonstrated the existence of two distinct anaerobic cellulose-fermenting organisms. The first breaks down cellulose to *hydrogen* and carbon dioxide, together with organic acids, among which were noted acetic, butyric and valeric acids, with traces of the higher fatty acids. The products of decomposition by the second

type of organism are *methane*, carbon dioxide and acetic and butyric acids. Kellermann and McBeth(6) claimed that the organisms described by Omelianski were not pure cultures and demonstrated further that cellulose is decomposed under aerobic conditions. They isolated 36 active species from various sources, all of which rapidly decomposed cellulose and other carbohydrates with the production of organic acids *but no gas*. More recently Viljoen, Fred and Peterson(7) have isolated in pure culture a thermophilic organism which destroyed cellulose at 65° C. forming mainly acetic acid, together with small amounts of butyric acid, ethyl alcohol, carbon dioxide and hydrogen. The action of cultures of such thermophilic cellulose fermenters on the crude fibre of different feeding stuffs is being studied in this Institute at the present time, with the object of ascertaining whether such a reaction may not form the basis of a method for the artificial determination of the digestibility of the fibrous constituent of feeding stuffs.

Before accepting the theory that cellulose is rendered available to the animal organism by bacterial activity in the rumen, of the nature of that described in the preceding paragraph, it will be well to consider the nutritive value to the animal of the products of such decomposition. The gaseous products, namely carbon dioxide, methane and hydrogen, are to be regarded as entirely valueless, since they are excreted as waste products from the body. Indeed, if, as is probable, this breakdown of fibre is primarily for the purpose of furnishing energy to the micro-organisms concerned, then it would appear that the latter are themselves incapable of utilising the whole of the energy of the cellulose molecule, since methane and hydrogen are oxidisable substances. Of the products of such breakdown, only the organic acids, chiefly acetic and butyric acids, can be supposed to be of any value to the animal. What constitutes their exact value for production purposes in the animal is not clear, though it is certain that they are much inferior in this respect to dextrose, the digestion product of starch. Since organic acids are capable of being oxidised in the body, it would follow that they may serve as sources of energy to the organism. It is generally assumed that lactic(8) and butyric(9) acids, when introduced directly into the circulation of the *fasting* animal, are able to protect body fat from oxidation and are able to replace, for this purpose, other nutrients in isodynamic proportions. It would appear, however, that acetic acid(10) is inferior to lactic and butyric acids in this respect.

Sufficient has been said to show that the theory of cellulose digestion, involving bacterial decomposition, in the rumen, of the cellulose complex

to methane, hydrogen, carbon dioxide and organic acids, is quite incapable of accounting for the findings of Kellner as to the value of digestible fibre in the fattening of ruminants. The products of such breakdown do not in any sense possess the productive value associated with the digestion product of starch. Such decomposition must simply be regarded as an incidental phase of activity in the rumen of the animal, whereby the bacteria furnish themselves, from cellulose and soluble carbohydrate alike, with a sufficiency of energy for their own metabolic purposes.

If the theory of cellulose digestion is to be brought into line with Kellner's findings, there appears to be no alternative but to assume that, under the influence of bacteria, the cellulose molecule undergoes hydrolytic cleavage to dextrose, or to a sugar capable of being hydrolysed further to dextrose. By such a process of breakdown, the cellulose would ultimately be absorbed into the blood stream in the form of dextrose, and the equal values for fattening purposes possessed by digestible fibre and digestible carbohydrate would be capable of simple explanation.

Let it be assumed then that the primary action of the cellulose-dissolving bacteria in the rumen consists in the hydrolysis of the cellulose complex to dextrose or some related sugar. There is no reason to suppose that the sugar so formed from cellulose will be further broken down to gases and organic acids to any greater extent than are the soluble carbohydrates originally present in the food. It has already been stated that on an average about 8 per cent. of the digestible nitrogen-free extractives of the food undergoes such destruction. The amount is obviously not only determined by the energy requirements of the micro-organisms, but also by the length of stay of the food in the rumen, and further by the fact that such activity is circumscribed by the resulting increasing acidity of the contents of the rumen. If, then, it can be assumed that sugar is formed as a primary product of the activity of the cellulose-splitting bacteria, then only about 8 per cent. of such sugar will undergo further bacterial breakdown to methane, hydrogen, carbon dioxide and organic acids, whilst the remainder will be available for absorption into the organism in the form of dextrose. It would follow, therefore, that every gm. of fibre so digested would yield to the organism as much dextrose as would a gm. of starch, and by this means Kellner's practical finding would be explained.

The tenability of such a hypothesis, however, depends upon the production of evidence to support the view that dextrose arises as an intermediate product when cultures of cellulose-fermenting bacteria are brought into contact with cellulose in artificial media. Such evidence is

forthcoming from the results of investigations carried out by Pringsheim<sup>(11)</sup>. The latter, by adding an antiseptic like toluene to stop the fermentation of filter paper by cellulose-splitting micro-organisms when the reaction was at its height, succeeded in isolating from the reaction mixture not only the disaccharose cellobiose, but also its product of further hydrolysis, namely dextrose. Under the conditions commonly obtaining in such experiments, where the medium is kept neutral by the presence of chalk, these sugars would rapidly be attacked by the bacteria and further broken down into gases and organic acids. It is not surprising, therefore, that numerous investigators have studied this reaction and failed to note the transient formation of dextrose. A mere examination of the end products of a reaction has frequently led to an erroneous conception of the nature of the reaction. The results obtained by Pringsheim afford convincing proof of the conception that in the destructive fermentation of cellulose, the initial change brought about by the bacteria is hydrolytic in character, resulting in the transformation of the cellulose complex into the sugars cellobiose and dextrose.

Cellobiose (glucose- $\beta$ -glucoside) when pure is a fine crystalline powder with a faintly sweet taste. It is much less soluble than cane sugar, reduces Fehling's solution and forms an osazone. It undergoes hydrolysis with the formation of dextrose. It is hydrolysed by emulsin but not by maltase. Takadiastase<sup>(12)</sup> contains a cellobiase which at 37° C. hydrolyses cellobiose completely to dextrose in three days. Malt extract<sup>(13)</sup>, in addition to the diastatic enzyme which hydrolyses starch to maltose, contains also a cellobiase and is able to hydrolyse cellobiose to dextrose at 37° C. at pH 5.

Presumably no cellobiase enzyme has been shown to be present in the digestive secretions of the ruminant animal. It must therefore be assumed that the cellobiose which arises from the cellulose in the rumen is capable of being hydrolysed further to dextrose by the activity of the bacteria themselves. In other words, the production of cellobiose represents the first stage in the bacterial hydrolysis of cellulose; the disaccharose is then promptly hydrolysed to dextrose in the second stage of the action. It will be noted, therefore, that a striking similarity exists between the mode of digestion of starch by enzymes and that of digestion of cellulose by bacteria. The resemblance is brought out in the following scheme:

Enzyme digestion:

Starch  $\rightarrow$  maltose (glucose- $\alpha$ -glucoside)  $\rightarrow$  dextrose.

Bacterial digestion:

Cellulose  $\rightarrow$  cellobiose (glucose- $\beta$ -glucoside)  $\rightarrow$  dextrose.

The foregoing attempt to propound a theory in connection with the digestion of cellulose in the ruminant organism which shall be compatible with Kellner's findings as to the productive value of digested fibre will focus attention on several problems of practical interest, among which may be mentioned: 1. The value of fodders, like young pasture grass, which contain fibre of a highly digestible character. 2. The desirability of cutting grass at an early stage for hay, at a time when the fibrous constituent is still unmixed with large amounts of indigestible ligno-cellulose. 3. The necessity for studying in detail the results of German war-time investigations into methods of treating straw with a view to increasing the digestibility of its crude fibre.

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# THE VARIATIONS IN MILK YIELDS CAUSED BY SEASON OF THE YEAR, SERVICE, AGE, AND DRY PERIOD, AND THEIR ELIMINATION.

## PART I. SEASON OF THE YEAR.

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(With Fourteen Text-figures.)

### INTRODUCTION.

ALTHOUGH the primary object of recording cows' milk yields is to provide a means of comparing one individual with another, it is not satisfactorily achieved when the records have been obtained, since yields are influenced to a large extent by environmental factors which vary from cow to cow. The lactation record is the result of two sets of factors—genetic and environmental—and for purposes of selection and breeding it is important to be able to make accurate allowance for the one, so as to arrive at a good estimate of the other.

Leaving aside the variation due to feeding and management (which, whilst undoubtedly large, is minimised for the cows of the same herd, and which it is hardly possible to study statistically in the existing data) the chief factors operating on the lactation yield (*i.e.* the measurable environmental factors) are the following:

(1) Season of the year; the lactation yield is influenced to a certain extent by the month of the year in which the cow calves.

(2) Service; *i.e.* the stage of the lactation at which the cow again becomes pregnant. The interval between calving and the next fertile service is here termed the Service Period (S.P.); thus if a cow calves on June 1st, and becomes pregnant again on July 1st, her S.P. for that lactation is 30 days.

(3) Age of the cow; unfortunately with ordinary commercial herds it is often—in fact usually—impossible to ascertain the exact age of a cow; it is possible, however, to find out in the vast majority of cases, how many times she has calved—consequently in this study age is measured in lactations; since, on the average, heifers calve for the first time at  $2\frac{1}{2}$  to 3 years of age, and then (as will be seen below to be the case) calve

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again at yearly intervals, the results can readily be interpreted to read the approximate age, by adding two on to the number of the lactation.

(4) Dry Period (D.P.)—or the period of rest (here measured in days), which the cow had before the commencement of the lactation with which we are concerned.

The effects of these four factors have already been studied in the data accumulated by the Penrith (Cumberland and North Westmorland) Milk Recording Society (3), (5)<sup>1</sup>, and the present paper gives the results of a statistical analysis, on the same lines, of the records of the Norfolk Milk Recording Society. These two districts were selected as being, perhaps, as opposite, as regards the conditions under which cows are kept, as any in this country; Penrith is essentially a grassland district, whereas Norfolk is predominantly arable, and a district in which there is a much more plentiful and varied supply of homegrown succulent food (roots etc.) for winter feeding. Perhaps the greatest difference between the two districts is that Norfolk Dairy Farmers look for their returns to the sale of milk, and consequently have a very real inducement to making the conditions optimum for its production, whilst in the Penrith district the returns are obtained from the sale of cows (for which there is a great demand), the disposal of milk being often rather a problem, and the chief value of a large record is the rather indirect one that it puts something on to the price of the cow and her relations.

Owing to the variation from farm to farm it is impossible to give typical rations throughout the year for the two districts, that could be regarded as useful guides; the chief differences that could be noticed are the following:

(a) In the Penrith district grass looms much larger on the menu—in consequence of this we find that by far the largest number of cows calve in spring, whereas in Norfolk autumn calving is rather the rule.

(b) In a few cases in Norfolk (it is suggested below that this practice ought to be much more common) catch-crops are grown to supplement the grass in July, August and September, whilst in the Penrith district this is almost unheard of; it must be remembered, though, that in the latter case the rainfall is very much greater.

(c) Roots are fed very heavily in Norfolk during winter, but are limited by the small area grown round Penrith.

<sup>1</sup> The numbers refer to the list of references at the end. See reference (3) for a fairly complete bibliography and discussion of other workers' results. These are not repeated in the present paper.

(d) The practice of feeding the cake ration according to the yield is the rule in Norfolk, but at any rate when the records studied were made, was the exception in the Penrith district, consequently, the rations in the former approached much more closely to the scientific requirement.

(e) The normal temperature is about 2° F. higher in Norfolk throughout the year (except in January, when it is slightly lower), and the growing season begins from a fortnight to a month earlier in the spring.

Throughout the country there appears to be a relation between the proportion of arable land and the average milk yield. In England Milk Recording Societies were operating in 27 counties for the five years 1818-19 to 1922-23, and the average yearly yield per cow for these was published by the Ministry of Agriculture. Hammond and Sanders(4) arranged these 27 counties in order of their five years average milk yield, and found that from the top to the bottom of the list there was a definite trend from the arable areas to those in which the proportion of grass was highest; in this list Norfolk came 3rd with an average of 6993 lb. and Penrith 26th with one of 5723 lb.

Table I shows the correlation—for these 27 counties—between the five years average yearly yield and the ratio, acres arable/acres grass, with

Table I. *Relation between 5 years average milk yield and ratio acres arable/acres grass. 27 English counties.*

5 yrs (1918-19 —1922-23) average milk yield (lb.)	Acres arable/acres grass																			Total
	0.20-0.39	0.40-0.59	0.60-0.79	0.80-0.99	1.00-1.19	1.20-1.39	1.40-1.59	1.60-1.79	1.80-1.99	2.00-2.19	2.20-2.39	2.40-2.59	2.60-2.79	2.80-2.99	3.00-3.19	3.20-3.39	3.40-3.59	3.60-3.79	3.80-3.99	
5600-5699	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
5700-5799	.	<i>P</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
5800-5899	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
5900-5999	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6000-6099	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6100-6199	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6200-6299	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
6300-6399	.	2	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4
6400-6499	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
6500-6599	1	.	2	.	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	5
6600-6699	1	2	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
6700-6799	.	.	.	1	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	2
6800-6899	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	1
6900-6999	.	.	.	.	.	.	.	.	.	.	.	.	.	.	<i>N</i>	.	.	.	.	1
7000-7099	.	.	.	.	.	.	.	.	1	.	1	.	.	.	.	.	.	.	.	2
Total	4	8	5	2	2	.	1	.	1	.	1	.	.	.	2	.	.	1	.	27

the pairs of values for Penrith and Norfolk as P. and N. respectively; it will be seen that these two are at about the opposite extremes in each case.

From the Table the correlation coefficient calculated is +.584 ± .086,



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and whilst it is not proposed to lay much stress on a coefficient based on only 27 observations, the value is large compared to its probable error, and does seem to indicate that there is some positive relationship between high proportion of arable land and high milk yield; it will be seen that there are exceptions—notably Derbyshire with an average of 6657 lb. and a ratio of only 0.27; Buckinghamshire, too, must be regarded as exceptional, with a very high milk yield and low arable proportion, though it could not be included in Table I as yield figures are not available until the year 1920–21; possibly the extensive use of brewers' grains in this county may be responsible for the high average, since many of the herds are maintained by buying rather than breeding, and consequently farmers are not deterred by the detrimental effect which brewers' grains are believed to have on the reproductive powers.

In collecting the data for this study the annual registers (L. 14) were obtained from each farmer, and all complete lactations were copied out, except those in which

- (a) The cow did not become pregnant again.
- (b) The cow was sucking a calf or calves for more than ten days.
- (c) There was any illness.

The following details were entered:

- (a) Herd letter, number and breed of cow.
- (b) Total lactation yield.
- (c) Number of days dry before the lactation.
- (d) Age of the cow—*i.e.* in lactations.
- (e) Date of calving.
- (f) Date of next fertile service.

(g) Service period—*i.e.* interval between (e) and (f); and then in columns the yield of milk for each week throughout the lactation.

In order to facilitate the working out of average lactation curves for cows calving at different seasons of the year, a separate sheet (or sheets) was kept for cows calving in each month of the year.

All the cows in the Penrith district were shorthorns or "shorthorn type," but records of a variety of breeds were obtained from Norfolk, and a separate set of sheets was kept for each breed.

Table II gives the number of lactations obtained for this study, according to month of calving and breed; it will be seen that sufficient records of pedigree Red Polls, Lincoln Reds and Friesians were included to allow of their being studied separately on some points. By far the greatest group is that labelled Mongrels; in common parlance there is a derogatory implication in this word, but nothing of the sort is intended

here, for, as will be seen later, this group gave the highest yield of all except Friesians—the word is used to distinguish from first cross Friesians etc. and to denote the ordinary non-pedigree type of cow prevalent in this country. In some cases doubt arose as to the breed of a particular cow—when this was so it was always entered in the “lower pedigree” group. Of pedigree Shorthorns, non-pedigree Shorthorns (foundation cows) Friesian crosses (first cross), Jerseys, Guernseys and Park cows, there were insufficient to give any results by themselves; they are included throughout, though, with the others under the heading “all cows.”

Table II. *Numbers of lactations obtained.*

Month of Calving	Mongrels	Red Polls	Lincoln Reds	Friesians	Friesian crosses	Non-pedigree Shorthorns	Pedigree Shorthorns	Park cattle	Jerseys and Guernseys	Totals
January	150	59	56	33	30	29	16	4	4	381
February	175	61	50	38	30	24	7	5	1	391
March	161	57	35	27	15	16	13	4	4	332
April	138	36	27	33	18	21	11	7	1	292
May	92	48	20	22	20	12	14	2	2	232
June	87	32	24	19	27	8	4	3	4	208
July	117	40	34	29	42	13	6	5	1	287
August	88	56	33	27	22	19	4	5	9	263
September	112	72	48	34	13	18	3	7	5	312
October	194	70	53	36	28	22	6	4	2	415
November	171	65	43	42	42	23	7	12	1	406
December	176	46	53	43	28	23	13	13	4	399
Total	1661	642	476	383	315	228	104	71	38	3918

In dealing with the effects of the four factors enumerated above, three comparisons have been made—between the two very dissimilar districts, Norfolk and Penrith, between the breeds, Friesians, Red Polls, Lincoln Reds and the Mongrel group, and between high and low yielding individuals. It may be said at once, that with the exception of the seasonal effect (which differs slightly in the two districts) no marked difference has been found in the effects of these four factors—*i.e.* only one table of standardising corrections is necessary.

The ordinary methods used by statisticians have been employed; the probable error of the mean  $\left( \cdot 67449 \cdot \frac{\sigma}{\sqrt{n}} \right)$  has been used, rather than the standard error, as having a rather simpler and more definite meaning, and an isolated difference has not been regarded as significant, unless it exceeded four times its own probable error—giving odds of 142 to 1 against the occurrence of such a deviation as a fluctuation of sampling. Sheppard's correction for grouping has not been applied to standard deviations.

## THE FACTORS STUDIED.

The problem attacked here, reduced as it is to only four of the many factors operating on the yield, is still a problem of multiple correlation, so that it would be well at the outset, to consider the distributions of these factors and their inter-relations.

The distribution of the 3918 lactations according to the month of calving has already been given (Table II) and calls for little comment. It will be seen that the largest number of cows calve in autumn and the smallest number in summer—thus October calvers are nearly twice as numerous as June calvers; the figure for July is rather high, probably because a certain proportion of the herds supply seaside districts and so need a large output during the holiday months. There seems to be no difference between the breeds in this respect, where the numbers are sufficient to give any regularity.

The distribution of the length of service period, for all cows, is given in the form of a frequency polygon in Fig 1. The mean length of service

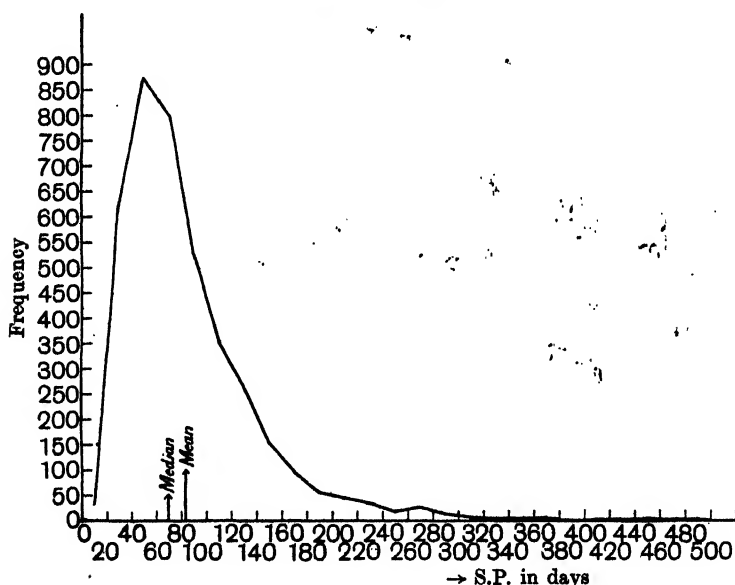


Fig. 1. Distribution of S.P. (all cows).

period was 83.5 days—which means that, on the average, these cows produced calves at intervals of almost exactly a year; the mode, however, lies at about 50 days, the mean being raised by a small number of cows that were left empty for a very long time. Table III gives the means,

medians and standard deviations for the distribution by breeds; although there is some variation the maximum difference between breeds is only a fortnight.

Table III. *Length of service period (days).*

	Mean	Median	Standard deviation
Mongrels	79.5 $\pm$ 0.8	67.4 $\pm$ 1.0	48.3 $\pm$ 0.6
Red Polls	82.9 $\pm$ 1.5	69.0 $\pm$ 1.9	56.9 $\pm$ 1.1
Lincoln Reds	87.7 $\pm$ 1.8	73.9 $\pm$ 2.3	59.3 $\pm$ 1.3
Friesians	93.3 $\pm$ 2.1	76.7 $\pm$ 2.6	61.4 $\pm$ 1.5
All Cows	83.5 $\pm$ 0.6	69.9 $\pm$ 0.8	54.3 $\pm$ 0.4

The distribution according to age for the different breeds is given in Table IV, as percentages for comparison; the figures for Penrith are also included to show the great difference in this respect—the sale of cows from that district leading to a much greater proportion of younger animals. Red Polls include a higher proportion of old cows—a fact which supports the claim to longevity made for this breed—but otherwise there are no marked breed differences; the mean number of lactations through which a cow lives in Norfolk is only 3.27.

Table IV. *Percentage distribution of age.*

Age	Mongrels	Red Polls	Lincoln Reds	Friesians	All cows (Norfolk)	Penrith
1st Lactation	23.1	21.9	22.8	24.5	23.5	34.3
2nd "	21.1	20.6	19.0	22.0	20.9	28.5
3rd "	17.4	18.4	16.1	16.1	17.6	18.1
4th "	14.0	12.9	14.0	14.5	13.6	10.3
5th "	9.9	7.3	11.4	10.5	9.6	5.1
6th "	6.3	6.3	6.8	5.7	6.1	1.9
7th "	4.2	4.1	5.9	3.0	4.2	.9
8th "	2.4	3.2	2.8	1.6	2.3	.7
9th "	.9	2.4	.8	1.6	1.2	.15
10th "	.4	1.3	.2	.5	.5	—
11th "	.25	.6	—	—	.3	.05
12th "	.05	.3	—	—	.1	—
13th "	—	.3	.2	—	.06	—
14th "	—	.2	—	—	.02	—
15th "	—	.2	—	—	.02	—
Total	100.0	100.0	100.0	100.0	100.0	100.0
Nos. of Lactations where age was unknown	114	7	3	11	170	8

The distribution of length of dry period (all cows) is represented in Fig. 2; the mean lengths of dry period for the different breeds were

Mongrels 51.3  $\pm$  0.69 days (1167 observations).

Red Polls 48.3  $\pm$  1.36 days (441 observations).

Lincoln Reds 67.8  $\pm$  1.57 days (334 observations).

Friesians 55.4  $\pm$  1.70 days (255 observations).

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Red Polls give a low mean and Lincoln Reds a markedly high one—in other words the former tend to milk on nearly up to the next calving time, whilst the latter dry off much sooner, a fact which is borne out by the average lactation curves given below. The distribution of this variable is very skew (skewness = + 0.686) the mean being 54.4 days and the mode only 28.0 days (calculated by the rough formula mode = mean - 3 (mean - median)—giving in this case a value agreeing fairly well with the peak of the curve).

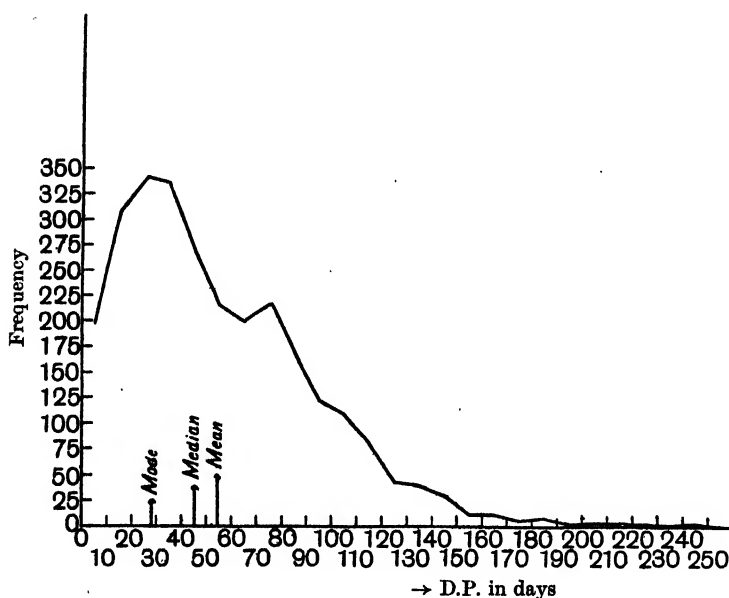


Fig. 2. Distribution of Dry Period (all cows).  
(One observation falling in the Interval "320-329 days" not shown.)

Apart from any physiological interest in the relations between these four factors, it is essential that any that exist should be realised, so that allowance may be made for them; thus though the actual significance of any one of these relationships may be doubtful, its existence in these particular data must be known before any sound criticism can be made, either of the results of this study, or of the methods by which they have been obtained.

Table  $\text{FV}$  gives the mean service and dry periods according to the month of calving; although the variation is not large the month of calving seems to have a slight influence in each case.

The mean S.P.'s are short in the case of cows calving in the months

February to June and above the normal for the others; since roughly half of these cows became pregnant again within about two months of calving, this means that the easiest time to get cows served successfully is from April to August. Hammond (2) has found that there is a seasonal variation in the intervals between the heat periods, in the number of hours on heat, and in the intensity of its manifestation; although the

Table V. *Variation of service and dry periods with month of calving.*

Month of calving	Mean service period (Days)	Mean dry period (Days)
January	85.7 $\pm$ 2.0	56.1 $\pm$ 1.6
February	82.7 $\pm$ 1.7	55.8 $\pm$ 1.5
March	82.3 $\pm$ 2.0	54.4 $\pm$ 1.7
April	78.5 $\pm$ 2.2	55.4 $\pm$ 2.0
May	78.5 $\pm$ 2.4	49.2 $\pm$ 2.0
June	75.9 $\pm$ 2.4	57.9 $\pm$ 2.3
July	84.7 $\pm$ 2.4	49.0 $\pm$ 1.6
August	83.4 $\pm$ 2.5	48.0 $\pm$ 1.8
September	87.6 $\pm$ 2.3	47.0 $\pm$ 1.5
October	86.7 $\pm$ 1.6	51.0 $\pm$ 1.5
November	83.2 $\pm$ 1.6	60.0 $\pm$ 1.5
December	87.7 $\pm$ 1.7	63.7 $\pm$ 1.6
Mean of the 12 months	83.1	54.0

intervals between the heat periods are longer in summer than in winter, the actual length of heat is much shorter in winter—often only seven or eight hours; under these conditions the cow is very liable to be missed, especially as during cold weather she shows less signs of heat; his results would, therefore, appear to provide an adequate explanation of this seasonal variation in S.P.

The D.P. on the average, is short for cows calving from July to October; this may in part be due to the occurrence of the spring “flush” of milk which has the effect of adding a few weeks on to the previous lactation of some of the cows calving in summer; this variation shows points of similarity with the seasonal variation in S.P., and, since cows with long S.P.’s tend to dry off at longer intervals before the next calving, the monthly variation in D.P. may depend to a certain extent on that in S.P. (*i.e.* for the previous year).

In these data there was also a relation between the age of the cow and the month of calving, for whereas the percentage of first calf heifers included in the records varied from 15 to 21 for the months of calving January to July, from August to December it lay between 23 and 37 with very high figures for September and October. It is, then, obvious that in many of these herds the practice is for heifers to calve in autumn,

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and allowance must be made for this in determining the effect of the month of calving on the lactation yield.

There was a relationship existing between the length of the S.P. and the age of the cows; thus for 879 first calf heifers the mean S.P. was  $89.9 \pm 1.33$  days; for 2542 records of the 2nd, 3rd, 4th, 5th and 6th lactations the mean S.P. was  $80.5 \pm 0.73$  days; for 156 records of the 7th lactation the mean S.P. was  $92.7 \pm 3.05$  days; and for 171 records of older cows the mean S.P. was  $95.7 \pm 3.01$  days.

The S.P. falls definitely from the 1st to 2nd lactation and rises again after the 6th lactation; during the 1st lactation service is often deliberately postponed to avoid stunting growth (note also the large proportion calving in September and October) and the rise in the case of old cows might be taken as indicating that senility, even in its early stages, has an adverse influence on the reproductive function; excluding 1st calvers, the correlation coefficient between S.P. and age was, however, only  $+0.065 \pm 0.013$  ( $\gamma = 0.108 \pm 0.012$ ) and consequently much stress must not be laid on the latter. This relationship has a not inconsiderable effect on the results obtained in studying age changes in yield.

No relation existed in these data between the lengths of the S.P. and of the preceding D.P. ( $r = +0.0165 \pm 0.013$ ), nor between age and the length of the D.P. ( $r = +0.061 \pm 0.013$ —this positive value was due to the shortness of the D.P.'s preceding the 2nd lactation).

The following facts must therefore be taken into account:

- (a) S.P.'s were short for cows calving from February to June.
- (b) D.P.'s were short for cows calving from July to October (also May).
- (c) The records for cows calving from August to December contained an abnormally high proportion of 1st calvers.
- (d) S.P.'s of 1st calvers were relatively long; they were short for lactations 2-6 and then longer again with later lactations.
- (e) D.P.'s were short before the 2nd lactation.

Whatever the causes of these, the method of analysing the data should be such that the minimum error is introduced by them; since the regressions do not even approach the linear, the partial correlation method is unsuitable, and that adopted, was to study one variation, find corrections to eliminate it, eliminate it, and proceed to the next.

Thus the variation in yield according to the month of calving was first found, and eliminating corrections obtained; S.P. was next studied and corrections worked out to allow for its length; these were then applied to the month of calving figures and new corrections found for the latter, which were not subject to the error introduced by the seasonal

variation in S.P. (these "corrected corrections" only differed slightly from the original ones). After applying the two sets of corrections for month of calving and S.P. to each lactation yield, the variation of yield with age was found, and the month of calving figures again adjusted, to allow for the relationship (c) above; having eliminated the other three it was possible to measure the effect of the length of the D.P., and finally each lactation was standardised in respect of S.P., age and D.P. and the seasonal variation worked out afresh.

This method of procedure is not above criticism, for if data sufficed they ought to be analysed simultaneously by all four factors; such a complete analysis, however, is clearly impossible—especially since it will be seen later, that age and D.P. must be studied by considering individual cows; evidence will be produced to show (it is thought, fairly satisfactorily) that the difficulties inherent in the relationships (a) to (e) have not affected the results.

It is interesting to note that the statements (a), (b) and (d), above, applied exactly to the Penrith data, as well as to the present ones; whilst (b) is probably due to (a) and to the effect of spring grazing, (a) and (d) appear more purely physiological in nature, as indicating that the reproductive vitality (like milk flow) is very sensitive to environment—that is, that successful service is more easily accomplished under the warmer and less artificial conditions of summer, and that the reproductive function reaches its maximum efficiency at about four years old and begins to decline from eight years onwards.

#### THE LENGTH OF THE LACTATION.

The distribution of length of lactation for all cows is given in Fig. 3; the mean was found to be 42.82 weeks (median = 41.48 weeks) and standard deviation 7.75 weeks.

This distribution is fairly symmetrical except for a small number of very long lactations—the longest being one week under two years; it is not however a "normal" distribution, the frequencies round the mean being increased at the expense of the shoulders of the curve.

Table VI gives the means, standard deviations and coefficients of variation obtained from the distributions of length of lactation according to breeds; as would be expected, and as will be seen below, the length of lactation depends very largely on the stage of the lactation at which the cow becomes pregnant again, so that in this table the mean times of service are also included. S.P.'s are, of course, measured from the actual



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date of calving, but the lactation only begins (officially) four days later; consequently Mongrels with a mean S.P. of 79.5 days are served at the  $\frac{79.5 - 4}{7} = 10.79$ th week of lactation. In the last column this figure is subtracted from the mean length of lactation to show for how long after service the various breeds remain in milk.

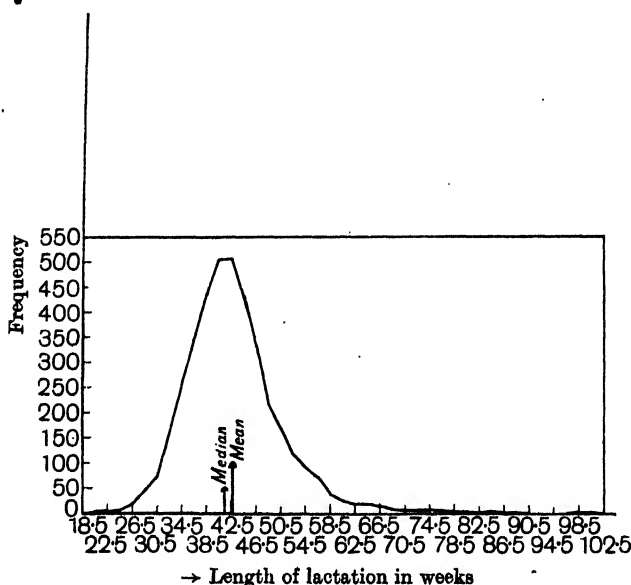


Fig. 3. Distribution of length of lactation (all cows).

Table VI. *Constants for length of lactation.*

Breeds	Length of lactation			Mean week of service	Weeks milking during pregnancy
	Mean (weeks)	Standard deviation (weeks)	Coefficient of variation		
Mongrels	42.56 $\pm$ 0.12	7.15 $\pm$ 0.08	16.80 $\pm$ 0.20	10.79	31.77
Red Polls	43.71 $\pm$ 0.22	8.20 $\pm$ 0.15	18.76 $\pm$ 0.37	11.27	32.44
Lincoln Reds	41.31 $\pm$ 0.26	8.27 $\pm$ 0.18	20.01 $\pm$ 0.46	11.96	29.35
Friesians	43.98 $\pm$ 0.28	8.03 $\pm$ 0.20	18.26 $\pm$ 0.46	12.76	31.22
All cows	42.82 $\pm$ 0.08	7.75 $\pm$ 0.06	18.10 $\pm$ 0.14	11.36	31.46

The actual differences between the breeds are not very great; but they are large compared to the probable errors. Although Friesians give the highest average the last column shows that this is accounted for by the larger mean S.P. for this breed. When S.P. is allowed for we see that Red Polls have a comparatively long lactation, whilst Lincoln Reds on the

other hand have a short one, Mongrels and Friesians agreeing very closely with the general mean for all cows.

From correlation tables drawn up between the length of the lactation and the length of the S.P. the following results were obtained:

	$r$	$\eta$	Regression equation. Length of lactation =
1st Calvers	$+0.795 \pm 0.008$	$0.811 \pm 0.008$	$35.70 + 0.105 \text{ s.p.}$
Others	$+0.758 \pm 0.005$	$0.780 \pm 0.005$	$33.30 + 0.109 \text{ s.p.}$

Length of lactation being measured in weeks and S.P. in days.

The correlation coefficient is slightly higher for 1st calvers, but the difference is not quite significant, and the close agreement between  $r$  and  $\eta$  in each case demonstrates the linearity of the regressions, whose equations are given; the regressions are shown in Fig. 4, and it will be seen that the points obtained lie very close to them.

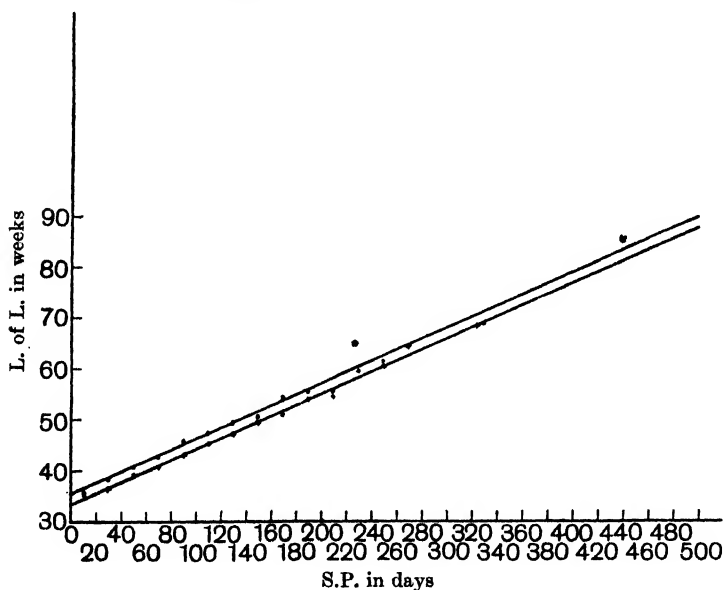


Fig. 4. Regression of length of lactation on S.P.

1st Calvers ——— Pts. obtained ●  
Others - - - - - Pts. obtained +

The above correlation coefficients rather overestimate the physiological relationship between these two variables, as some few cases occur of very long S.P.'s being associated with only moderately long lactations, and these are often excluded automatically from the data—that is, the farmer would sell the cow before she was pregnant again, and hence her

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lactation record would be excluded as having no S.P.; such cases were, however, rare.

The chief interest of Fig. 4 is the remarkably constant increase in length of lactation for 1st calvers, over the whole range of S.P.; the regression lines are practically parallel, heifers milking for approximately two weeks longer than older cows with the same S.P.—whatever that S.P. may be. This longer lactation of the 1st calf heifer accounts, of course, for the fact already noted that in these data the D.P. preceding the 2nd lactation was abnormally short.

It is interesting to see what these regressions have to tell as to how long a cow milks after service, when this occurs at different stages of the lactation; the figures for Table VII were obtained from the regression equation for cows other than heifers, but it is clear the regression would show the same thing for heifers. The second column of this table gives the stage of the lactation at which frequency commences, with the S.P. given in the first column (*e.g.* (20–4) days = 2·3 weeks); the third column gives the mean length of lactation associated with this S.P. (*i.e.* from the regression) and the last column was obtained by subtracting the second from the third.

Table VII.

S.P.	Stage of lactation at which served (weeks)	Length of lactation ( <i>i.e.</i> 33·30 + 0·109 S.P. (weeks)	Weeks in milk during pregnancy (weeks)
20 Days	2·3	35·4	33·1
120 "	16·6	46·3	29·7
220 "	30·9	57·2	26·3
320 "	45·1	68·1	23·0
420 "	59·4	79·0	19·6

A cow served three weeks after calving will, on the average, keep on milking for the first 33 weeks of pregnancy, but one not served till 60 weeks after calving will usually be dry 20 weeks later—in other words, though the length of the lactation is very highly dependent on the time of service, it shows a marked “regression” to the normal period of 42 weeks.

If 1st calvers be excluded, the regressions between (1) length of lactation, (2) S.P., (3) age, (4) D.P., all approximate to the linear form, and consequently may be treated by the partial correlation method. The following regression equation was obtained:

$$X_1 = 35.0 + 0.763 X_2 + 0.118 X_3 - 0.281 X_4.$$

This shows that the effect of age is negligible (*i.e.* when 1st calvers are excluded), that roughly three weeks are added on to the lactation for

every four weeks' delay in service, and that the lactation is about one week shorter for every four weeks the cow was dry before its commencement; the latter may not have any physiological significance, but may demonstrate the probability that if a cow was dry for a considerable time before calving, she will again dry off early.

By using the regression equations between length of lactation and S.P. it was possible to ascertain the variation in length of lactation according to month of calving, under constant conditions as regards S.P.; the result was negative, there being no significant deviations for the several months.

#### SEASON OF THE YEAR.

##### SECTION A: NORFOLK (ALL COWS) AND PENRITH.

##### *Total lactation yield.*

The more important constants found from the distributions of the total lactation yield for all cows calving in the several calendar months are given in Table VIII.

Table VIII. *Effect of month of calving on the total lactation yield (uncorrected).*

##### *All Norfolk Cows.*

Month of calving	Mean total lactation yield lb.	Median lb.	Approximate mode*	$\sigma$ lb.	Sk.	V
Jan.	7081 $\pm$ 69	6808 $\pm$ 86	6262	1995 $\pm$ 49	+ 411 $\pm$ .034	28.17 $\pm$ .74
Feb.	7218 $\pm$ 68	6969 $\pm$ 85	6471	1994 $\pm$ 48	+ 375 $\pm$ .035	27.63 $\pm$ .71
March	6953 $\pm$ 77	6758 $\pm$ 96	6400	2069 $\pm$ 54	+ 260 $\pm$ .041	29.83 $\pm$ .85
April	6993 $\pm$ 81	6705 $\pm$ 101	6129	2050 $\pm$ 57	+ 421 $\pm$ .039	29.32 $\pm$ .88
May	6649 $\pm$ 89	6397 $\pm$ 111	5893	2007 $\pm$ 63	+ 377 $\pm$ .045	30.18 $\pm$ 1.03
June	6389 $\pm$ 93	6045 $\pm$ 117	5357	1990 $\pm$ 66	+ 519 $\pm$ .043	31.15 $\pm$ 1.12
July	6567 $\pm$ 80	6380 $\pm$ 100	6006	2012 $\pm$ 57	+ 279 $\pm$ .043	30.64 $\pm$ .94
Aug.	6522 $\pm$ 90	6394 $\pm$ 113	6138	2162 $\pm$ 64	+ 178 $\pm$ .049	33.15 $\pm$ 1.08
Sept.	6667 $\pm$ 74	6542 $\pm$ 92	6292	1928 $\pm$ 52	+ 195 $\pm$ .044	28.92 $\pm$ .84
Oct.	7099 $\pm$ 69	6776 $\pm$ 86	6130	2078 $\pm$ 49	+ 466 $\pm$ .032	29.27 $\pm$ .74
Nov.	7111 $\pm$ 70	6924 $\pm$ 88	6550	2089 $\pm$ 49	+ 267 $\pm$ .037	29.38 $\pm$ .75
Dec.	7375 $\pm$ 77	7019 $\pm$ 96	6307	2274 $\pm$ 54	+ 470 $\pm$ .032	30.83 $\pm$ .80
Mean of all months	6885					

\* Mode = Mean - 3 (Mean - Median).

It will be seen that the mode lies considerably below the mean giving positive values of skewness between 0.178 and 0.519, which do not seem to vary in any definite way with the different months. The standard deviation fluctuates round about 2000 lb. with no very large departure from that value; the coefficient of variation varies between 27 and 33.

The variation of the total lactation yield with the month of calving,

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although small in amount, appears quite definite—the means obtained are below the average of 6885 lb. for all the months May to September inclusive, and above for the other seven months.

These means, however, are open to criticism as to their representation of this relationship, for we have seen that S.P.'s, D.P.'s and age, all vary from month to month. By the corrections obtained in this work it was possible to allow for these other factors; each lactation yield was corrected separately for the other three variables, and new distributions of the corrected yields were drawn up; although this is somewhat anticipating results to be given later, the means obtained from these corrected yields are given in Table IX.

Table IX. *Effect of month of calving on lactation yield (corrected).*

Month of calving	Mean corrected lactation yield lb.	Effect of correction in raising (+) or lessening (-) mean lb.	Correction for month of calving %
January	6950 ± 61	- 131	- 0.9
February	7084 ± 61	- 134	- 2.8
March	6900 ± 71	- 53	- 0.2
April	7043 ± 74	+ 50	- 2.2
May	6661 ± 78	+ 12	+ 3.4
June	6437 ± 84	+ 48	+ 7.0
July	6559 ± 71	- 8	+ 5.0
August	6687 ± 81	+ 165	+ 3.0
September	6855 ± 67	+ 188	+ 0.5
October	7223 ± 64	+ 124	- 4.7
November	7067 ± 63	- 44	- 2.6
December	7154 ± 62	- 221	- 3.8

In the first column of this table is shown the mean corrected total lactation yield; the object of the corrections is to estimate from each lactation what the mature yield of the cow would be under certain standard conditions, and consequently their application gives a figure some 13-14 per cent. higher than the original one; thus the mean of all the monthly corrected means was 7848 lb. To enable an easy comparison to be made with the uncorrected mean, therefore, each figure was multiplied by  $\frac{6885}{7848}$  to bring the series down to the original level. The next

column shows the effect of the corrections in raising or lowering the monthly means; the figures for August, September, and October are noticeably increased because of the high proportion of first calf heifers included, which has already been noted; the means for the winter months are lowered, as on the average, these cows have long S.P.'s and D.P.'s, and April, May and June are put up by allowing for the short S.P.'s associated with cows calving then.

It will be seen that the probable errors of the corrected means are less than those of the uncorrected ones, and it seems certain that the means in Table IX gives a much truer representation of the effect of the month of calving on the total lactation yield, than do those of Table VIII.

In the last column the corrections to eliminate this variation are given; taking as our standard the mean of all months (which corresponds very closely to the yields for March and September), a percentage must be subtracted from the yields of all cows calving between October and April, and a percentage added to those of cows calving in the summer. The actual corrections are not very large, varying as they do between + 7.0 per cent. and - 4.7 per cent.—a small range compared to that necessary to allow for the other three factors considered in this paper.

In Norfolk, the figures show, the best time for a cow to calve for a high yield is from October to February, and this is brought out very clearly in Fig 5, which represents the mean corrected yields for the

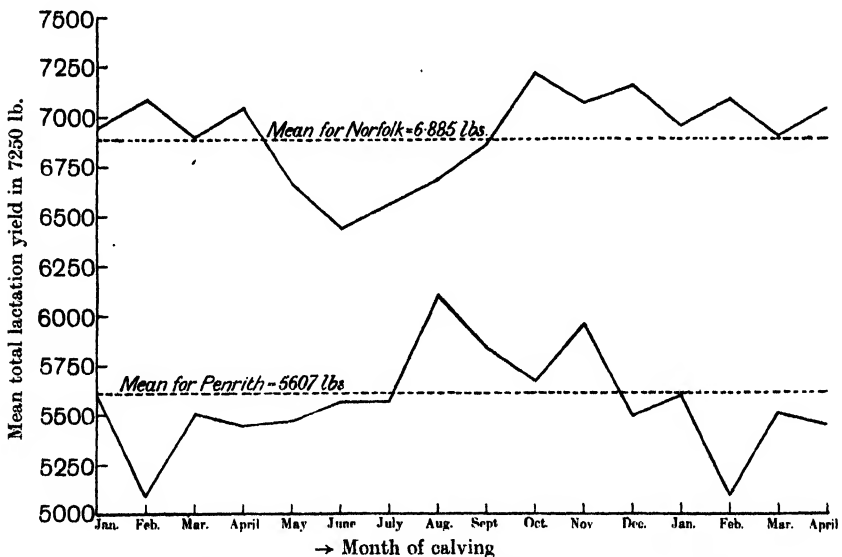


Fig. 5. Variation of the total lactation yield with the month of calving. (Corrected means.)

various months of calving; the first four months are repeated at the right of the figure to give a continuous picture of the variation, and the corresponding results from the Penrith data (already reported (3)) are included for comparison.

The highest mean of all is given by October calvers and the figure

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then decreases uniformly till April; at May there is a sudden drop and the lowest point is given by June calvers; from these we get a steady rise till September and then a rather steep one up to October again. It is clearly indicated that the best months for calving are from October to February but there is some difficulty in deciding which is actually the optimum month for calving—with a very large mass of data the curve might rise sharply at October and then fall in approximately a straight line to April, or it might be rounded giving a maximum at December. Owing to the multiplicity of factors which go to form this seasonal effect, no attempt has been made to smooth out the results, because it is, perhaps, probable that the true function sought is not by any means smooth, and consequently such a procedure would be more likely to introduce errors than to eliminate them; from May to September however there is little need for any smoothing process, and, as regards the optimum, for practical purposes no greater precision is necessary than that the best months are October, November and December.

In view of the fairly smoothly running figures represented above, more reliance can be placed on the differences between the monthly means than is indicated by their probable errors; still some of these differences exceed four times their probable errors, and so may be regarded as significant apart from other evidence; this is shown in Table X.

This table really consists of two separate halves; with the same means it is obvious that the same figures would be obtained in the corresponding cells of the upper right and lower left portions of the table, with opposite signs. To economise space, therefore, the upper right half of the table was derived from the mean uncorrected yields for each month, and the lower left from corrected yields. Thus it is possible to see the significance of the difference between any two months, compared either by "raw" or corrected yields. For instance, to compare January and July: from the appropriate square in the top line it is seen that with "raw" means this difference is  $514 \pm 106$  lb. in favour of January, a difference equal to 4.8 times its probable error; with corrected means the cell in the first column shows a difference of  $391 \pm 94$  lb. or a difference equal to 4.2 times its probable error.

Taking the arbitrary criterion of four times the probable error as constituting a significant difference, all those showing significance are enclosed in black squares; although the probable errors are smaller with corrected means, some significance is lost, showing that, over all the months, the gross effect of the other three factors tends to make the seasonal variation more than it really is.

Table X. *Significance of the differences between the monthly means. Uncorrected means.*

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Jan.	—	-137 ± 97 1.4	+144 ± 103 1.4	+88 ± 106 0.8	+432 ± 112 3.9	+682 ± 116 6.0	-514 ± 106 4.6	-559 ± 113 4.9	+414 ± 101 4.1	-18 ± 97 0.2	-30 ± 98 0.3	-294 ± 103 2.8
Feb.	+134 ± 86 1.5	—	+261 ± 102 2.7	+225 ± 106 2.1	+569 ± 112 5.1	+829 ± 115 7.2	+651 ± 105 6.2	+696 ± 113 6.2	+551 ± 100 5.5	+219 ± 97 2.3	+107 ± 98 1.1	-157 ± 103 1.5
Mar.	-50 ± 94 0.5	-184 ± 94 2.0	—	-56 ± 111 0.5	-288 ± 117 2.5	-548 ± 121 4.5	+370 ± 111 3.3	+415 ± 118 3.5	+270 ± 106 2.5	-162 ± 103 1.6	-174 ± 104 1.7	-438 ± 108 4.1
April	+93 ± 96 1.0	-41 ± 96 0.4	+143 ± 103 1.4	—	-344 ± 120 2.9	-604 ± 123 4.9	+426 ± 114 3.7	+471 ± 121 3.9	+326 ± 109 3.0	-106 ± 106 1.0	-118 ± 107 1.1	-382 ± 112 3.4
May	-289 ± 99 2.9	-423 ± 99 4.3	-239 ± 105 2.3	-382 ± 108 3.5	—	+260 ± 129 2.0	+82 ± 120 0.7	+127 ± 126 1.0	-18 ± 116 0.2	-450 ± 112 4.0	-462 ± 113 4.1	-726 ± 112 6.5
June	-513 ± 104 4.9	-647 ± 104 6.2	-463 ± 110 4.2	-606 ± 112 5.4	-224 ± 115 1.9	—	-178 ± 113 1.6	-133 ± 129 1.0	-278 ± 119 2.3	-710 ± 113 6.1	-722 ± 116 6.2	-986 ± 121 8.1
July	-391 ± 94 4.2	-525 ± 94 5.6	-341 ± 100 3.4	-484 ± 103 4.7	-102 ± 105 1.0	+122 ± 110 1.1	—	+45 ± 120 0.4	-100 ± 109 0.9	-532 ± 106 5.0	-544 ± 103 5.1	-808 ± 111 7.3
Aug.	-263 ± 101 2.6	-397 ± 101 3.9	-213 ± 108 2.0	-356 ± 110 3.2	+26 ± 112 0.2	+250 ± 117 2.1	+128 ± 108 1.2	—	-145 ± 116 1.3	-577 ± 113 5.1	-589 ± 114 5.2	-853 ± 118 7.2
Sept.	-95 ± 91 1.0	-270 ± 91 3.0	-45 ± 98 0.5	-188 ± 100 1.9	+194 ± 103 1.9	+418 ± 107 3.9	+296 ± 98 3.0	+168 ± 105 1.0	—	-432 ± 101 4.3	-444 ± 101 4.4	-708 ± 106 6.7
Oct.	+273 ± 88 3.1	+139 ± 86 1.6	+323 ± 96 3.4	+180 ± 97 1.9	+562 ± 101 5.6	+787 ± 106 7.4	+664 ± 96 6.9	-536 ± 103 5.2	+368 ± 93 4.0	—	-12 ± 98 0.1	-276 ± 103 2.7
Nov.	+117 ± 88 1.3	-17 ± 88 0.2	+167 ± 95 1.8	+23 ± 97 0.2	+406 ± 100 4.1	+630 ± 105 6.0	+508 ± 95 5.3	-380 ± 103 3.7	-212 ± 92 2.3	-156 ± 90 1.7	—	-264 ± 104 2.5
Dec.	+204 ± 87 2.3	+70 ± 87 0.8	+254 ± 94 2.7	+111 ± 97 1.1	+493 ± 100 4.9	+717 ± 104 6.9	+595 ± 94 6.3	+467 ± 102 4.6	+299 ± 91 3.3	-99 ± 89 0.8	+87 ± 88 1.0	—

*Corrected means.*



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The two halves of the table have, however, very much the same tale to tell. In each case there are two blocks of significant differences; roughly speaking, all the differences between the good calving months October to February on the one hand, and the bad calving months May to August on the other hand, are significant, whether we consider mean "raw" or corrected yields. It is interesting to see that with both sets of means the drop from April to June, and the rise from September to October, are both equal to, or greater than, four times their probable errors, whilst the difference between April and May is 2.9 times (uncorrected) and 3.5 times (corrected) its probable error.

For the yields associated with the various months of calving we have, then, a fairly smoothly running set of figures showing a definite trend; the individual differences between the high and low months are nearly all significant, and the fall from April to June, and the rise from September to October, are significant by themselves. Under these circumstances it is impossible to doubt that the relation is as illustrated in Fig. 5. The only objection that could reasonably be raised against this conclusion, would be that it may be that the best cows, or best managed herds, tend to calve in autumn, and the worst in summer; no evidence has been found of this, and as 108 different herds provided the data for this study, herd differences in this respect might be supposed to be neutralised. Such an objection, however, cannot be answered directly, and so average lactation curves have been prepared of all the cows calving in the several months; it will be seen that these provide a complete explanation of the variation found above.

Turning for a moment to the figures for Penrith, the chief divergence is that the high months come from August to November—*i.e.* two months earlier—and the Penrith figure for February is very low; in passing we shall see that this divergence also is explained by a comparison of the average lactation curves for the two districts.

#### LACTATION CURVES.

Three of the twelve mean lactation curves for cows calving in various months of the year, are given in Figs. 6, 7 and 8—those for February, May and October calvers; the continuous line represents the curve for Norfolk cows and the broken one that for Penrith. Small crosses appear on each curve at the mean date of service, and the calendar months given above show the time of year throughout the lactation; the horizontals corresponding to yields of 100 and 200 lb. per week are included to facilitate comparison.

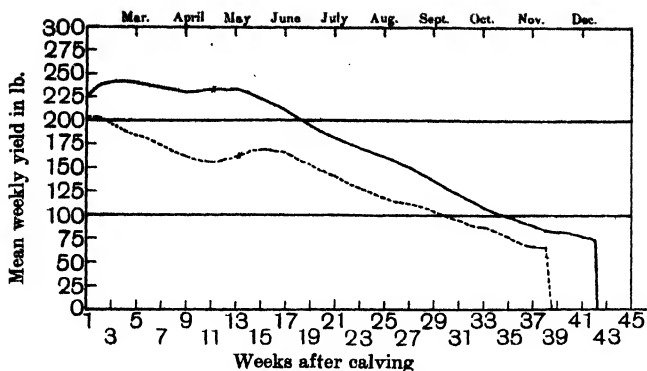


Fig. 6. February calvers.

Norfolk ——— No. of lactations = 391; Mean total lactation yield = 7218 lb.  
 Penrith ..... No. of lactations = 125; Mean total lactation yield = 5169 lb.

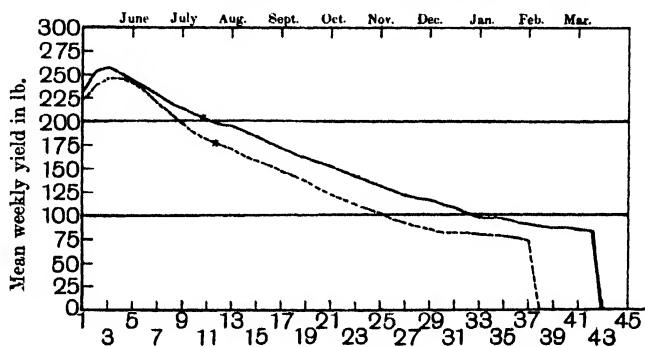


Fig. 7. May calvers.

Norfolk ——— No. of lactations = 232; Mean total lactation yield = 6649 lb.  
 Penrith ..... No. of lactations = 122; Mean total lactation yield = 5343 lb.

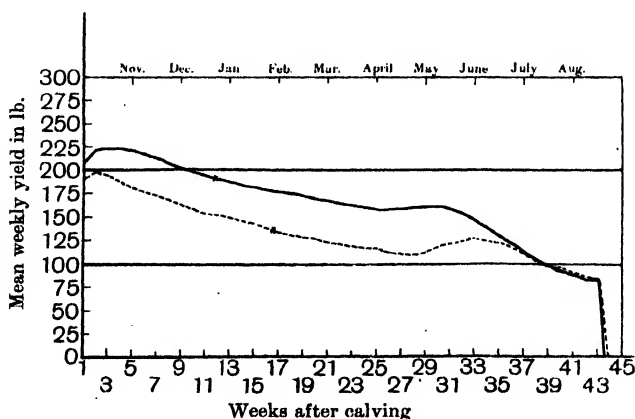


Fig. 8. October calvers.

Norfolk ——— No. of lactations = 415; Mean total lactation yield = 7099 lb.  
 Penrith ..... No. of lactations = 106; Mean total lactation yield = 5677 lb.

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These curves have not been smoothed in any way; in every case it will be seen that the numbers included were quite sufficient to obliterate individual fluctuations—in fact experience shows that as few as 20 lactations, or even less, are enough for this, until some of them begin to dry off.

In order to test the significance of the difference in the levels of any two curves the probable errors of the mean weekly yields must be known.

Approximately, the coefficients of variation of weekly yields for cows calving at the same time of the year, were found to be 25 for weeks 1–5, 26 for weeks 6–15, 28 for weeks 16–25, and then to rise up to about 40 as cows begin to dry off.

If then, for example, we wish to compare the yield during the 3rd week of the lactation of September and October calvers (Norfolk) the means for which are respectively 214 and 224 lb. (numbers of lactations 312 and 415) we have:

For September calvers the standard deviation

$$= \frac{214}{100} \times 25 = 53.5 \text{ lb.}$$

$$\text{Probable Error of the Mean} = .67449 \times \frac{53.5}{\sqrt{312}} = 2.04 \text{ lb.}$$

Similarly for October calvers. Probable error of mean = 1.85 lb.

$$\text{i.e. P.E. of diff.} = \sqrt{(2.04)^2 + (1.85)^2} = 2.75.$$

The difference is therefore  $10.0 \pm 2.75$  lb. in favour of October—a difference equal to 3.6 times its P.E.; whilst this is not quite significant according to our criterion, it shows that the odds are fairly heavily against this difference being due to chance, and therefore that the probability is high that there are factors operating which result in October calvers commencing their lactation at a higher level than do September calvers.

*Norfolk.* The series of 12 lactation curves (of which three are given) shows a steady and continuous variation between the level type of curve for October calvers and the steep form for May calvers. The main points of importance are brought out in Figs. 9 and 10 which illustrate the mean yield and the mean fall in yield during each month. To obtain these each monthly lactation curve was split up into nine periods of four weeks, agreeing as closely as possible with the first nine calendar months of the lactation—one week being excluded every three months to allow for the odd days; in this way there were nine figures for each calendar month taken at exactly corresponding stages of the lactation, from 9 different

lactation curves. In order, therefore, to find out how the yield of milk varies throughout the year, the yield during each of these four weekly periods was totalled and divided by four, and the nine different values so found for each month averaged—with the result shown in Fig. 9. This figure represents, what would be the variation in the actual output of milk throughout the year, from a very large herd of cows that were calving down regularly all the time. Fig. 10 shows the variation in the rate at which yields fall during the different months; in this case the yield in the first week of each four-weekly period was subtracted from that in the last, to give the rise (+) or fall (–) in three weeks; this figure was then divided by three, and the nine values for each month averaged; in this case the figures for Penrith are shown on a separate scale to prevent overlapping.

Fig. 9 shows that in Norfolk the most milk is given during May and from thence it falls off fairly regularly till November and then rises again—the yield being above the average, for the months April to August and below during autumn and winter. The rate of fall of yield (Fig. 10) might be taken as an (inverse) index of management; the fall is very rapid during June and July, and September and October, suggesting that it is at these two periods that there is the greatest scope for improvement; the figures show very clearly how the young growing grass in spring puts the yield up, but they indicate that by June it is declining in value, and that insufficient allowance is made for this.

In this respect the results obtained by Woodman, Blunt and Stewart<sup>(6)</sup> are of interest. They find that the dry matter of grazed pasture grass in the early part of the season possesses a nutritive value comparable with that of concentrates like linseed cake and barley meal, and is much superior to that of the best quality of meadow hay. The value diminishes somewhat during the dry mid season, falling roughly 16 per cent. of its spring value. With the rains of late summer and autumn the nutritive value rises gradually to attain a figure at the end of September, not far short of the spring value; during the cold weather of late October and early November the value begins to fall off again. The percentage of digestible protein remains high throughout, falling slightly in the dry mid-season, but recovering to its spring level towards the end of the season. These results were of course obtained on a pasture whose herbage was kept short by frequent cutting; in many cases bad grazing allows grass to mature when it becomes much less digestible.

Under ordinary farm conditions Fig 10 shows that the drop as the grass gets older is very great and this considerably lowers the lactation

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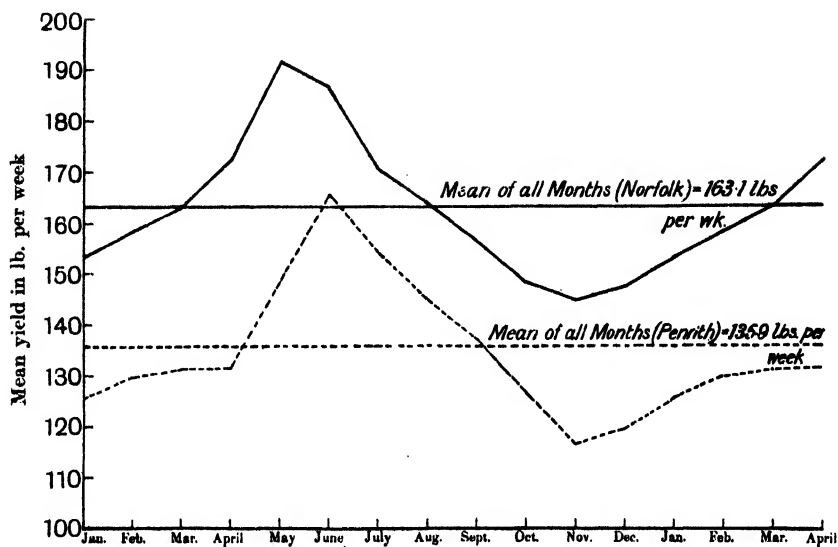


Fig. 9. Mean yield during different months of the year.

Norfolk —————  
Penrith - - - - -

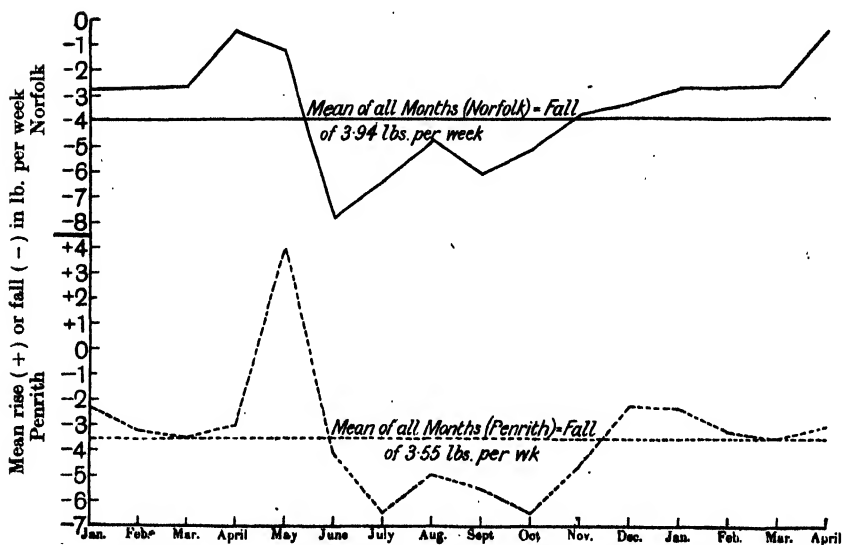


Fig. 10. Mean rate of fall of yield during different months of the year.

Norfolk —————  
Penrith - - - - -

yield; there would appear to be three methods of attempting to arrest this fall—the better and more thorough grazing of pastures during summer to keep the herbage young, the growing of a succession of catch crops for feeding green during June and July, and the feeding of cake to supplement the grass as its nutritive value declines. Up to a certain point, of course, this latter is commonly practised, but these figures suggest that more skill is needed in estimating the value of grass, and particularly that the rapid fall in its value from May onwards is not generally recognised. It is admittedly difficult to assess pasture at its true worth by inspection, but the variations in its quality are faithfully reflected in the milk records (for the “lag” in response to variations in it, would not appear to exceed a few days), and it should not be impossible to adjust the cake ration so that the total feed is commensurate with the yield. Perhaps in many cases the farmer is not unduly distressed by a large fall in yield during June, as he often has difficulty in disposing of his surplus milk during the flush period, but it is quite clear from these results, that better management at this time of the year would have a marked effect in increasing the average yearly yield from the herd, and in counteracting the shortage often experienced in July and August, following droughts; more especially is this desirable where a seaside district is supplied, and a greater output needed in the holiday months.

After this period the index of management rises steadily all through the autumn and winter with the exception of September and October; these two months are at the change from summer to winter conditions, and although the grass is growing very little, no other succulent food is available in many cases; there is a possibility of providing it, however, in the form of white turnips, cabbage, maize or silage.

Fig. 9 is something like that obtained by plotting the normal temperature for each month, with the modification that the maximum and minimum come two months earlier for yield than for temperature; if there is any connection between temperature and milk flow it would appear to be that the flow is increased as the temperature rises, and vice versa, but not that the temperature itself is of any importance, since the hottest months are July and August. It is not thought that this relationship is a very close one, but the largest fall in temperature is from September to November, and it is possible that the fall in yields at that period may in part be due to fall in temperature.

The primary object of working out these lactation curves was to find the explanation of the variation in the total lactation yield with the

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month of calving (Fig. 5), and the points that have been touched on provide this explanation.

Taking October calvers first (the highest yielders) we have seen that their curve is a very flat one; their flow is maintained well throughout the winter and they reap considerable benefit from the spring flush; after that the bad period in June and July soon dries them off. Going on to November and December calvers we have two considerations—the June drop comes earlier and so tends to lower the yield, but cows come into milk at a higher level and not having fallen for so long make more gain during the spring.

These two tendencies are accentuated in the following months (January to April) and as the time of high yield before, and including, the spring flush gets shorter, lower yields would be expected. Furthermore, from January onwards another point must be considered, namely that the second period of rapid fall (September and October) is beginning to play a part; with January calvers this only affects the last few weeks of the lactation, but when we get to April calvers it has by no means an inconsiderable effect.

Taking the winter months, therefore, the curves would suggest that October, November, and December calvers have the best chance of making high records under ordinary farming conditions, and that from January a gradual decline would be expected until we get to May. With a few minor fluctuations this expectation is fulfilled in Fig. 5 and the only difficulty would appear to be to see why April calvers should give a mean higher than March calvers; it is possible that this may be due to “errors of sampling.”

The June calver commences her lactation at the worst period and consequently starts at a fairly low level, and falls off rapidly till November, and we should therefore expect a large drop from May to June. The curves for all the months June to September are all steep until November (*i.e.* a large fall during the second bad period in September and October) and then flatten out—as the flattening out comes earlier in the lactation the yield is raised. Finally October calvers come into milk after nearly all the period of rapid fall is past and so begin their lactation at a higher level than do September calvers—a difference which is maintained throughout; consequently it is not surprising to find in Fig. 5 a sudden rise from September to October.

Figs. 9 and 10 provide a fairly complete explanation of the variation portrayed in Fig. 5; it should be noticed, therefore, that these two figures were obtained from all the 3918 lactations included in this study—that

is from all the cows calving in all the 12 months of the year; they are, therefore, not subject to any error which might be introduced by any practice such as calving the best cows in autumn and the worst in summer. If such a practice existed, while it would not affect these figures it would greatly influence the variation in Fig. 5, and consequently the conclusions drawn from the first two and the last would not agree. That they do agree so closely seems proof positive that over all these herds no factor of this sort is affecting the results; hence we may safely conclude that Fig. 5 represents the variation concerned truly—*i.e.* the average seasonal variation in Norfolk; it will be realised that a certain amount of variation from farm to farm, according to the system of management, is probable.

One other point is rather striking and might be emphasised—namely, that this seasonal variation is due almost entirely to the variation in the rate of fall of yield from month to month. The low yield of early summer calvers is due to the rapid drop during summer, and if this drop could be arrested, there seems no reason why these cows should not give yields as high as autumn calvers; in fact it seems justifiable to conclude that the existence of this seasonal variation is evidence of bad management somewhere—and we have already remarked on the times of the year when this bad management is at its height—during summer. Yields are maintained best of all during the spring flush of grass and therefore the conditions seem to be optimum at that time; with more knowledge of foods and feeding it may be possible to get even better conditions than obtain then, but at present it would appear that the aim should be to imitate those conditions as far as possible throughout the year. It is interesting to note that Wylie(7) working with 2900 records of Register of Merit Jerseys found very much less seasonal variation than that found in these data; *i.e.* where feeding and management are on a very high plane the seasonal variation is almost obliterated.

It may be impossible to provide young growing grass from June to October, but a succession of catch crops would provide a continued supply of succulent food; it may be that more skilful cake feeding at this time would avert the loss of milk, or it may be that succulent food has a value over and above that indicated by the digestible nutrients it contains, in that the constituents of a young growing vegetable cell are more likely to contain the special substances in the proportions required for the production of milk, than are the constituents of the mature vegetable cell; at the present time this is a much debated question and one which, it appears, can only be satisfactorily decided by definite experiment.



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*Penrith.* If the two curves in each of Figs. 6, 7 and 8 be compared, the most striking difference is that the spring flush is very much more marked in Penrith—there it is a definite rise in the curve, whereas in the case of Norfolk it is little more than a temporary cessation of the fall; also it occurs roughly three weeks later in the north.

The summer fall in yield is approximately the same in Penrith but with the onset of winter the curves widen out; the difference between the two curves for February calvers is very great—whereas in Norfolk little fall takes place before the spring flush, in Penrith there is a very large drop.

The difference in the seasonal variations is brought out by comparing the ratios between the “outputs” (Fig. 9) in the various months for the two districts; these ratios are all above the average (1·20) for the months November to May, and below that average from June to October. The output of milk is, therefore, more uniform throughout the year in Norfolk than in Penrith—it would, of course, be expected that the variation would be accentuated in the Penrith district, where so much reliance is placed on grass; we see then that the conditions for winter milk production are relatively much better in Norfolk—is this due to a better root supply?

A comparison of the rates of fall in yield throughout the year, shows that this fall is markedly greater in Penrith during February, March and April (see Figs. 6 and 8).

We have then three main differences in the seasonal variation in these two districts.

(a) The yield is more uniform throughout the year in Norfolk, the winter conditions being much better.

(b) The conditions are (comparatively) very bad for milk production in the Penrith district from February to April (see Figs. 9 and 10).

(c) The spring flush is later in Penrith, and coming as it does after a time of very poor conditions, is very much accentuated.

It seems that (b) is the key to the difference in the effect of the month of calving in the two districts; if the bad period at the end of the winter (in Penrith) comes early in the lactation a low yield results, and consequently although autumn calvers still have flatter curves, and give higher yields, the best months for calving are moved further forward—that is, away from the period February to April.

The two curves in Fig. 5 are not therefore necessarily contradictory; they indicate that in arable districts the best months of calving are October to February, because the plentiful root supply enables yields to

be maintained well throughout the winter, but that in grassland districts the best time for calving comes earlier. In this respect it is interesting to note the results given by Zwagerman(\*) for Zeeland; this is an arable district and the best calving months are one month later still (*i.e.* November to March) with a maximum at December; in the same way the bad months come later, the lowest means being given by July and August calvers. It appears that the differences between districts in this respect are due to the differences in types of farming—*i.e.* proportion of arable land—and are not very largely due to differences in climate.

*Mean Curves.* Fig. 11 gives what may be termed the “mean curves” for the two districts; that is to say each curve is the mean of the 12 monthly lactation curves obtained—each week being averaged separately throughout; in these curves the seasonal variation is eliminated and they show the normal initial rise and subsequent fall of yield.

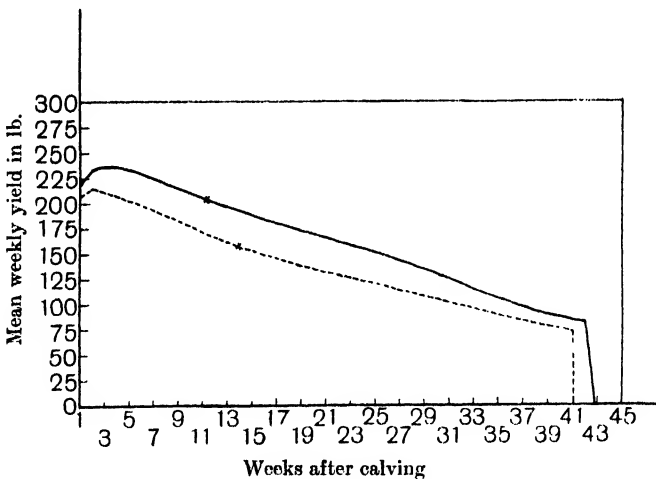


Fig. 11. Mean curves.

Norfolk ————— Mean total lactation yield = 6885 lb.; Mean first week yield = 219.4 lb.  
 Penrith - - - - - Mean total lactation yield = 5607 lb.; Mean first week yield = 207.1 lb.

The total lactation yield represented by the mean curve for Norfolk is 6885 lb. and that for Penrith is 5607 lb.; *i.e.* the Norfolk cows yield 22.8 per cent. more milk in the lactation than do the Penrith cows; but the two curves start at very much the same level, the mean yield in the first week being 219.4 lb. for Norfolk and 207.1 lb. for Penrith, or the Norfolk figure is 5.9 per cent. higher. The yield during the first week is subject to great variation, but with such large numbers as are here involved, the averages might be taken to give a fair comparison between

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the milking capabilities of the two sets of cows; if this be admitted we must conclude that of the 22·8 per cent. difference in favour of Norfolk not more than 6 per cent. is due to better cows, and approximately 17 per cent. to better conditions of feeding and management. Furthermore, the S.P. is shorter by nearly three weeks in Norfolk and this would reduce the yield by roughly 5 per cent. so that the conditions of Norfolk appear to be worth 22 per cent. more milk than those in the Penrith neighbourhood; this is still a conservative estimate, for the cows in the latter district were younger and consequently (cf. later) would be expected to have a flatter curve; we see however that the fall is much less rapid in Norfolk and the flow is maintained for over a week and a half longer, although pregnancy occurs 18 days earlier.

### SECTION B. BREEDS.

In section A we have considered the seasonal variations in milk yield in the light of the results obtained from all the cows included in the Norfolk and Penrith sets of data; in the former case there were a fairly large number of records of pedigree Red Polls, Lincoln Reds and Friesians, and here it is proposed to consider these separately in order to ascertain whether the conclusions apply with equal force to each breed; the large group of Mongrels is also included to form a sort of standard for comparison.

The mean total lactation yields (corrected) for the different months of calving for these four breeds are given in Table XI; with so few lactations

Table XI. *Effect of month of calving on lactation yield (corrected yields).*

Month of calving	Mongrels				Red Polls				Lincoln Reds				Friesians			
	Mean lactation yield (lbs.)	3-months weighted mean (lbs.)	Comparative yield		Mean lactation yield (lbs.)	3-months weighted mean (lbs.)	Comparative yield		Mean lactation yield (lbs.)	3-months weighted mean (lbs.)	Comparative yield		Mean lactation yield (lbs.)	3-months weighted mean (lbs.)	Comparative yield	
January	7165				6940				6431				7955			
February	7396	7300	104		6756	6767	100		6597	6501	101		7838	7766	100	
March	7320				6599				6474				7432			
April	7304				6470				6068				8130			
May	6637	6859	97		6726	6508	96		6975	6323	98		7338	7713	99	
June	6381				6224				6068				7423			
July	6646				5834				6386				7843			
August	6924	6766	96		6376	6538	97		6334	6292	98		7839	7930	102	
September	6767				7055				6196				8076			
October	7419				7212				6426				7689			
November	6948	7220	103		7696	7273	107		6534	6581	103		7395	7706	99	
December	7266				6770				6774				8023			

(see Table II) the probable errors involved are rather large and consequently the three-monthly weighted means have been worked out, and these have been calculated as percentages of the mean of all months, so that a ready comparison may be made.

There is a surprising agreement in these percentages for the first three breeds; for the winter months they vary from 100-104, for spring and summer 96-98, and for autumn 103-107; for all three breeds the figures are higher for autumn and winter than for spring and summer, and the extent of the variation is much the same, although with Lincoln Reds it appears to be slightly less. With Friesians, on the other hand, the results do not agree, the figures being remarkably constant; owing to paucity of data it would not however be justifiable to draw any definite conclusion from this.

Average monthly lactation curves for each breed separately were drawn up, but limitations of space forbid their inclusion; it may be said however that only two, minor, breed peculiarities were found.

The first was that there is a tendency for Red Polls to yield relatively well during summer and correspondingly poorly during winter, Friesians and Lincoln Reds showing a slight tendency in the opposite direction. Thus "output" curves (cf. Fig. 9) were calculated for the four breeds and that for Mongrels used as a standard for comparison; the mean monthly "output" for the other three breeds were put in the form of ratios to the mean "output" for the corresponding months with Mongrels; in the case of Red Polls these ratios were all above the average from May to August and below for the other months; with Friesians they were below the average from May to October and with Lincoln Reds they were low from April to July. The amount of the variation, however, was small (2-3 per cent.).

The second peculiarity is more definite and is illustrated in Fig. 12, which gives the mean curves (cf. Fig. 11) for Friesians, Lincoln Reds and Red Polls.

Although they start at different levels<sup>1</sup> the two curves for Friesians and Red Polls are very similar and terminate at about the same point. The curve for Lincoln Reds is, however, of a very different type; it commences at a point between the other two, but falls more rapidly, crossing the Red Poll curve at the 27th week; these cows, also, were dry over a fortnight earlier although the time of service was approximately the same.

<sup>1</sup> The comparison of the milking capabilities (total lactation yields) of the various breeds will be made later.

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The similarities in the behaviour of these breeds according to season, were, though, much more striking than these two peculiarities; this fact, in conjunction with Table XI, points to the conclusion that only one set

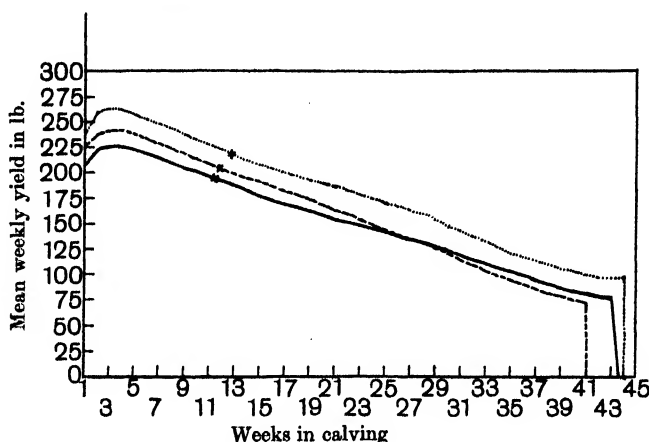


Fig. 12. Mean curves.

Friesians	.....	Mean total lactation yield = 7832 lb.
Red Polls	————	Mean total lactation yield = 6594 lb.
Lincoln Reds	- - - -	Mean total lactation yield = 6610 lb.

of standardising corrections is necessary for the month of calving and that it is not confined to any one breed, but is universally applicable; in the case of Friesians, perhaps, this should be regarded as not yet proved.

### SECTION C. HIGH AND LOW YIELDERS.

One other comparison must be made before leaving the subject of the seasonal variations in milk yield—namely that between high and low yielding individuals; do they vary in the same way and will the set of corrections given in Section A be as efficient for each class?

In studying this, one is confronted at the outset by what would appear to be a very minor difficulty, but which is in reality a rather formidable one—that is, the difficulty of discriminating between high and low yielders. Should it be done by dividing up the cows or the lactations? In the latter case it is quite possible for one individual to have one lactation among the low yielders, and another among the high yielders; there is, however, nothing essentially contradictory in this, for if during one lactation a cow comes up to the qualification for a high yielder there seems no reason to omit this lactation from the high yielding

group, whatever her previous or subsequent records may have been—during that lactation she was, at least temporarily, a high yielder and would be expected to be affected in the same way as other good producers.

The principle adopted was to split the distribution of lactations into three, and to take the top third as high yielders and the bottom third as low yielders, neglecting the middle third altogether; in this way only a very few individuals migrated from one of the groups included to the other, at different stages of their lives.

But another difficulty presents itself for there are undoubtedly two types of high yielders—there is the cow that gives a very high yield at first but soon falls off, and there is the persistent milker, that may not give a very high maximum, but keeps her flow up and so gives a large total in the lactation.

The problem was first attacked by discriminating on the basis of the maximum yield, because the corrections to allow for Service, etc., had not been worked out. The method was as follows: The work was restricted to the Mongrels, to keep the labour within reasonable limits and to avoid the two very small breed peculiarities that have been found; frequency distributions were drawn up of the maximum weekly yields of all the Mongrels calving in the different months of the year, and by interpolation the weekly yield for each month of calving ascertained that was exceeded by the maximum yield of one-third of the lactations and the one below which one-third lay. For instance, for October calvers the two figures found were 267 lb. and 215 lb.; all Mongrels calving in October, then, and giving a maximum yield of over 267 lb. in a week were taken as high yielders, and all those never reaching 215 lb. as low yielders; similarly for the other months of calving. Obviously it would be impossible to have the same figures for all months, for in that case the high yielders would consist largely of spring calvers and the low yielders of autumn calvers.

These have been called high and low (maximum) yielders, but the division is far from satisfactory; the greatest drawback to it is that first calvers rarely give a high maximum, and consequently a very large proportion of them went in with the low yielders; thus one heifer, calving in January, gave 11,454 lb. of milk in a not very abnormal lactation of 71 weeks, but her maximum yield was only 210 lb. in a week, and as the critical figure for January low yielders was 231 lb. she had to be included in them; this heifer was still yielding 182 lb. in the 67th week after calving, and, despite her low maximum, was obviously a very excellent milker.

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Two groups of cows were obtained, however, of which one was undoubtedly higher yielding than the other and average curves, etc., were worked out. Later, when the other three factors could be eliminated, each total lactation yield was corrected and the lactations in each month of calving split up into thirds (as before) on the basis of the corrected total yield—for instance of the January calvers all those giving a corrected figure of over 8653 lb. were taken as high yielders and all those less than 7205 lb. as low yielders. These are called high and low (total) yielders and the figures given in this section were obtained from them.

There were 552 lactations in each of the two groups, and these included the same number of cows calving in each month (*i.e.* in both groups there were 49 January calvers and 59 February calvers; in the case of a few months, as would be expected, the numbers did not quite agree—for March calvers there were 55 high yielders and 53 low yielders, but this was the greatest divergence).

The following means were found from the two groups:

	Service period (days)	Length of lactation (weeks)	Dry period (days)
High (total) yielders	82.1 $\pm$ 1.5	45.63 $\pm$ 0.21	36.9 $\pm$ 0.9
Low (total) yielders	78.3 $\pm$ 1.3	39.43 $\pm$ 0.18	63.9 $\pm$ 1.3
Difference	3.8 $\pm$ 2.0	6.20 $\pm$ 0.28	27.0 $\pm$ 1.6

The difference between the mean S.P.'s is slight and insignificant, but the mean length of lactation is 6.2 weeks greater for high yielders—a difference which is certainly significant; it would, of course, be expected that one of the distinctions between high and low (total) yielders would be that the former maintain their flow longer, and, as a natural corollary, that they were dry for a shorter time; this latter is also seen to be definitely the case from the mean D.P.'s.

The distribution of age was very similar in each group, except that there was a slight preponderance of old cows amongst the high yielders (due to "weeding out").

The mean corrected lactation yields for the two classes for each month of calving are represented in Fig. 13.

These two curves are obviously very similar; for the low yielders the means for the months May to September are below the average and those for October to April above, and the same is true for high yielders, with the solitary exception of November calvers. But the similarity is closer than that, for the difference between the highest and lowest months is 1431 lb. for high yielders and 882 lb. for low yielders; if these be divided by the respective means of all months (*i.e.* 8914 and 5254) the

figures 0.161 and 0.168 are given for the two groups—in other words the percentage variation is almost exactly the same.

It would be unwise to place much confidence on the slight differences between the curves, but it is perhaps worth noting, that the means for cows calving in spring are comparatively higher for high yielders. The

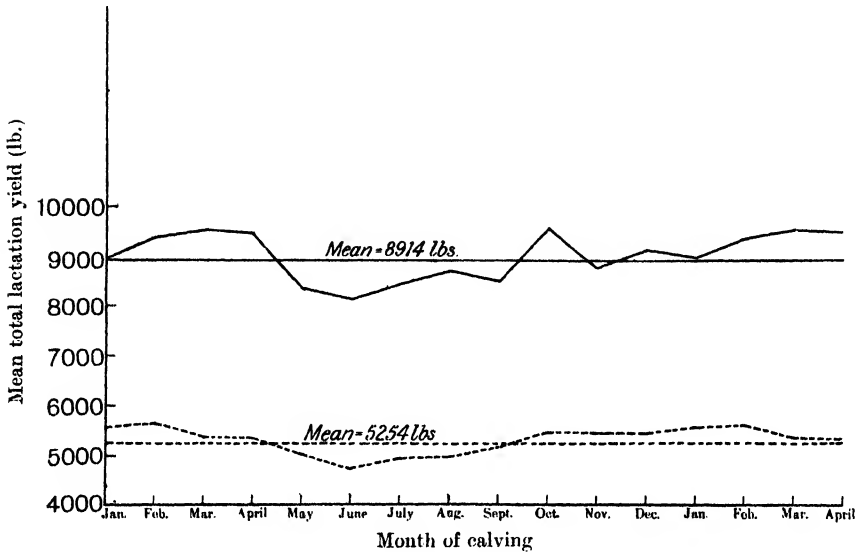


Fig. 13. Variation of total lactation yield with month of calving.  
High ——— and Low . . . . . (total) yielders.

conclusion to be drawn, though, is that the effect of the month of calving is *proportionately* the same with all cows, good, bad, or indifferent; that, for instance, the high means obtained in Section A for cows calving in autumn were not due to increased yields with one grade only, but to factors that acted to the same extent over all of them (in actual pounds, though, it will be realised that the gain in autumn calving is greater with high yielding cows).

From the mean monthly lactation curves that were calculated for each group Table XII has been drawn up—it gives the mean “output” and drop in yield (cf. Figs. 9 and 10) for each group and the ratios between them.

The figures for high yielders may be regarded as fairly reliable, but those for low yielders are subject to an error—introduced by the shortness of the lactation for that group; to get these results the first nine months after calving (40 weeks) are taken and the lactations of low yielders are



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usually ended by the 38th or 39th week; after the mean length of the lactation the curves were, of course, worked out as before until all the cows were dry, but with reduced numbers and cows drying off irregularly the figures do not run quite so smoothly. Consequently the figures for the drop are not so trustworthy, but those for the mean yield (mean of four weeks in each of the 108 periods) are not much affected; however the

Table. XII. *Mean weekly yield and drop in the different months—  
High and Low (total) yielders.*

	Mean weekly yield			Mean weekly drop		
	High yielders lb.	Low yielders lb.	High/Low yielders	High yielders lb.	Low yielders lb.	High/Low yielders
January	189.7	121.8	1.56	2.46	3.01	0.82
February	195.9	123.9	1.58	2.74	3.20	0.86
March	202.4	130.4	1.55	2.78	2.22	0.96
April	214.9	138.6	1.55	0.23	0.93	
May	236.5	153.9	1.54	1.58	1.60	0.99
June	229.5	155.1	1.48	8.36	6.69	1.25
July	211.1	142.0	1.49	6.57	5.92	1.11
August	204.4	135.5	1.51	5.02	4.63	1.08
September	196.5	128.4	1.53	6.38	6.11	1.04
October	185.9	120.0	1.55	5.98	4.92	1.21
November	181.8	116.5	1.56	4.22	3.60	1.21
December	182.3	117.5	1.55	3.56	3.11	1.14
Mean	202.6	132.0	1.535	4.16	3.82	1.09

only bad irregularity is round about the spring flush and it will be seen that for the drop the figures for March and April have been taken together

$$\left\{ \begin{array}{l} \frac{2.79 + .23}{2.22 + .93} = .96 \end{array} \right\}.$$

The ratios for mean weekly yield do not vary very appreciably and, remembering that a lactation occupies 9 or 10 months, they support the tentative conclusion already reached that the month of calving effect is the same for both groups. To be more precise, however, the ratios are below normal for the months June to September, *i.e.* during this period high yielders do not compare as favourably with low yielders.

The drop figures are low for the months January to May and August and September; as has been pointed out, these must be taken with reserve, but the fact that high yielders fall off relatively more rapidly during June and July seems very clear. Remembering that the variation in weekly yield shows the same thing, we must conclude that the bad conditions (be it feeding or weather, etc.) from June onwards, which have been discussed in Section A, make high yielders fall off more rapidly, not only in actual pounds of milk, but also proportionately to their yield.

This conclusion lends strong support to the view that the impairment of the feeding value of grass is responsible for the great fall in yield at this time of the year; such a result must then naturally follow. Indeed, the only surprising thing is that this is not more marked—for it might be asked, why should low yielders fall off at all abnormally, seeing that in June their average yield is only 155.1 lb. per week, or a little over two gallons a day? Where any real attempt is made to allow for the deterioration of pasture during the summer, the plan usually adopted is to allow, say, that the grass is sufficient for maintenance, and the production of four gallons of milk during May, three gallons during June, etc., and to feed cake to cows yielding more; the fact that low yielders also fall off very quickly during June, suggests that the reasoning behind this practice must be at fault.

Must we, then, fall back on the conclusion that it is not entirely the digestible nutrients contained in grass that must be thought of, but that there is also some beneficial effect due to those ill-defined characteristics usually embraced by the term "succulence"? If this is so we should expect good and bad cows to be affected in the same way—possibly equally.

Eckles and Reed<sup>(1)</sup> attempted to determine what the essential difference was between a high and a low yielding cow, and their conclusion was as follows: "A superior dairy cow is simply one with a large capacity for using food above the maintenance requirement, and one that uses this available food for milk production." This seems to provide an alternative explanation; it would indicate that as the grass gets less nutritious and more fibrous, a high yielder can cope with a sufficient bulk to provide the nutriment necessary, say, for the production of three gallons of milk, whereas the low yielder may not be able to obtain enough for two gallons. If this is so, it seems that our ideas in rationing must be modified somewhat, for rations are usually based on the assumption that a cow can only consume some 33 lb. of dry matter a day; on this basis it becomes a matter of some difficulty to feed sufficient for a six or seven gallon cow, but it would seem possible that the seven gallon cow is such, ultimately, because she is not limited to this 33 lb. of dry matter daily; individual peculiarities in this respect are now being realised by the most advanced feeders.

It is a common fault to argue at great length from few premises—a fault that has possibly been committed here; the problem is one that can only be settled by experiment, for it is realised that many factors are influencing these data, coming as they do, from a large variety of farms.

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But these results do indicate that grass during June and July may not be good enough even for the low yielder; that with her small capacity it is too fibrous a food to maintain even her output, and that, if a fall is to be avoided, all cows should be given a cake feed (possibly a reduced one) according to yield, before being allowed to fill themselves in the field. Perhaps an even better practice would be to supplement the pasture by a succession of catch crops fed in the young state—if any benefit is inherent in “succulence,” this would provide it.

Primarily the work described in this section was undertaken to ascertain whether the corrections found for the month of calving, were applicable to both high and low yielders; it is claimed that the results show that only one set of corrections (percentage) is necessary and that their efficacy is the same for all ordinary grades of cows, for the difference between these two groups was fairly wide. It might be added that exactly the same conclusions were drawn from high and low (maximum) yielders, but owing to the questionable nature of that division, the actual figures are not given; the agreement, however, provides an indication that age is not a factor that need be considered in this respect, for these high and low (maximum) yielders might be fairly accurately described as old and young cows.

Finally a little must be said about the mean curves for the two sets of cows, which are shown in Fig. 14.

To anyone interested in milk secretion the difference between the lactation curves of high and low producers is of fundamental importance, and from this figure it is possible to analyse this difference slightly. The increase in yield from the low to the high group is, of course, represented by the area between the curves, and this is due to three things—the fact that the high yielder's curve starts at a higher level, that it keeps up better (*i.e.* the ratio between the ordinates is greater at the end of the curve than at the beginning) and that it ends considerably later although the Service Period is only half a week longer.

From the curves we have the following:

	Total lactation yield lb.	Maximum weekly yield lb.	Total yield to 39th week lb.	Yield after the 39th week lb.
High (total) yielders	8888	273.06	8102	786
Low (total) yielders	5349	207.72	5349	—
Ratio High/Low	1.662	1.315	1.515	.147*

\* In proportion to the total yield of low yielders.

The ratios show that this group of high yielders gave 66.2 per cent. more milk than the low yielders; but the maximum yield is only 31.5 per

cent. more, so that it appears that only this amount is due to what we might describe as greater physiological capacity. By the 39th week the low yielders are dry and the others have given 8102 lb. or 51.5 per cent. more, and hence as a rough measure  $51.5 - 31.5 = 20.0$  per cent. of the

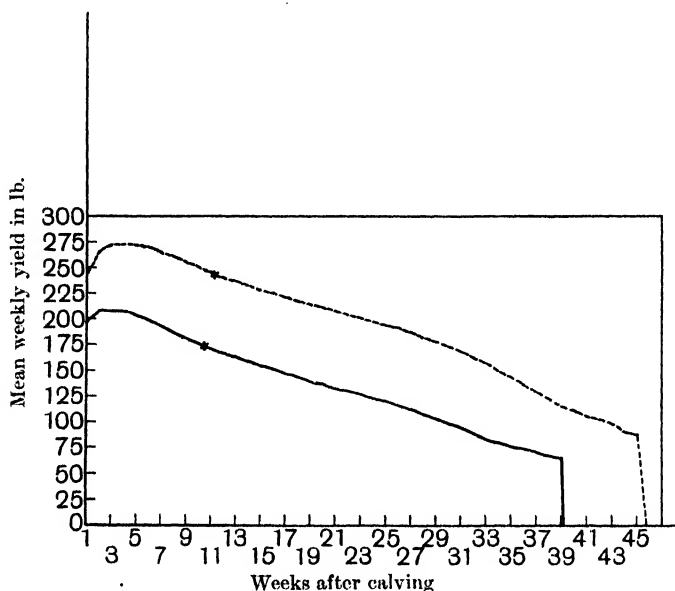


Fig. 14. Mean curves.

High (total) yielders - - - - - Mean total lactation yield = 8888 lb.; Maximum weekly yield = 273.06 lb.  
 Low (total) yielders ——— Mean total lactation yield = 5349 lb.; Maximum weekly yield = 207.72 lb.

difference is due to the fact that with high yielders the flow is kept up better; after the 39th week the high yielders give 786 lb. of milk or 14.7 per cent. of the total for the other group—this proportion is, then, directly due to the fact that they do not dry off so early.

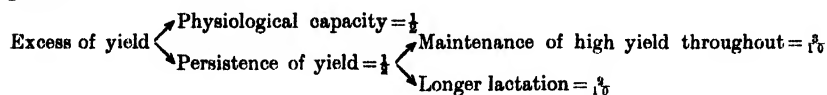
We must apportion the excess of yield, then, as follows:

To greater physiological capacity	- 31.5 %	= 47.6 % of the difference.
To better maintenance of yield	- 20.0 %	= 30.2 % of the difference.
To longer lactation	- 14.7 %	= 22.2 % of the difference.
	<u>66.2 %</u>	<u>100.0 %</u>

No very great accuracy is necessary, neither is it obtainable, for an indeterminate part of the more rapid fall of low yielders from about the 35th week onwards, is due to the fact that they are drying off—i.e. is due to the third factor; all that is wanted is an approximate estimate of the

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amounts due to each cause; in round figures the analysis might be represented as follows:



The maximum yield during a lactation, then, only tells one half of the story, and it will be apparent that it is untrustworthy as a basis for selection; in dividing up the Mongrel lactations into high and low yielders, we have, in fact, been selecting two groups of cows, on a large scale, so that it is relevant to mention the results obtained, when the division was done according to the maximum yield. The mean curves for high and low (maximum) yielders gave the following:

	Total lactation yield lb.	Maximum weekly yield lb.	Length of lactation
High (maximum) yielders	8490	303.56	42.38
Low (maximum) yielders	5513	176.68	42.34
Ratio High/Low	1.540	1.718	—

Here our difference is only 54 per cent. in the total yield, showing that our method of selection has not been so efficient; the length of lactation is exactly the same and the maximum yield is nearly 72 per cent. more for the high yielders. Although we have here selected two groups such that the physiological capacity of one is 72 per cent. (cf. 31.5 per cent. before) more than the other, the total yield is only 54 per cent. (cf. 66 per cent. before) higher, showing that instead of selecting as our best group the cows with great persistence of yield, we have picked out a group with a marked weakness in this respect. This suggests a definite danger that may exist in the selection for breeding, of animals, whose only qualification is a high maximum, and whose total yield is not given the attention it deserves.

If cows are selected from a district for high and low total yields, we have seen, that one half of the difference is due to what we have called physiological capacity and one half to persistence of yield; the question then naturally arises—how much of this latter is due to the cow herself and how much to her environment? In the Penrith records it was found (5) that persistence was a very definite individual characteristic, besides being subject to environment; a further analysis of this is eminently desirable, to show, if not how much is possible in the way of improving yields by management, at least how much difference is caused by management in practice; it is hoped to provide figures bearing on this at a later date.

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# THE NUTRITIVE VALUE OF DRIED SPENT HOPS.

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WITH THE COLLABORATION OF  
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SPENT hops is a minor by-product of the brewing industry which, in most cases, is disposed of in the wet state as an organic manure of low manurial value. But, when such material is dried, a bulky product is obtained which possesses a high absorptive capacity for water and can be used effectively as a "filler" to soak up important by-products from other industries such as molasses and treacle. The thin frail nature of the hop petals is still maintained and a very large surface per unit weight is exposed.

## *Absorptive capacity.*

The absorptive capacity may be illustrated by the fact that, after three hours' immersion in water, the dried material soaked up  $2\frac{1}{2}$  times its weight of water to give a product which was just moist, the same type of product after 24 hours' immersion having a moisture content of 78 per cent. The material could soak up 3 times its weight of water to give a fairly dry product. Well stirred with 3, 4, 5, and 6 times its weight of molasses containing 50 per cent. water, an attractive product which could easily be moulded into cubes was formed, although the product from 6 times its weight was softer and would flatten out with the pressure in bags, whereas the samples containing less molasses possessed greater physical strength which increased with keeping.

## *Analysis of the by-product.*

The use of this material as part of a feeding stuff demanded that the nutritive value of the material itself apart from that of what it had absorbed should be determined. The samples of dried spent hops obtained were analysed, the following being their average analysis:

Crude protein	Ether extract	Crude fibre	Nitrogen-free extractives	Moisture	Ash
21.51	8.33	25.03	33.10	5.84	6.19

The material showed a high crude protein and ether extract content for

this class of material, but the amount of crude fibre present was equal to that found in good meadow hay. The ash content was high.

The investigation of the feeding value consisted of the determination of the digestibilities of the various feeding constituents of the material together with a detailed examination of the ether extract.

#### *Digestibility determinations.*

Three 15 months-old Kent-Southdown wethers were procured by the Department as experimental animals for the digestibility determinations and were housed in properly constructed cages for the purpose. The harness used for collecting the excreta was of the modified Halnan type, consisting of a large rubber sheet firmly but comfortably strapped to the hindquarters of the animal, the faeces being collected in trays at the rear of each cage. The floor of each cage was lined with sheet lead sloping to the centre to a drainage hole, the urine being led off to bottles under the cages. The animals were fed from zinc-lined mangers, and plenty of water was available for each animal in an easily accessible trough.

The animals were brought in off grass (late June) and slowly got on to a dry food ration by adding to lawn clippings increasing quantities of meadow hay chaff and linseed cake, and after a week were fed solely on dry food. The amount of dried spent hops which the animals could cope with comfortably per day was then determined. At first the animals rejected the material mixed with hay and lump linseed cake completely, but after ten days of experimenting it was found that 100 gm. per head per day was the maximum which could be eaten with ease, and that amount had to be mixed thoroughly with linseed cake meal. The animals were then fed for a preliminary period of a week on the above amount mixed with the basal ration, and this period was followed by one of twelve days during which the faeces and urine were collected. The addition of spent hops to the basal ration then ceased, and after a preliminary period of seven days on the basal ration another period of twelve days ensued during which faeces and urine were again collected. The animals were then brought slowly back to green food and returned to the farm. Even this small amount of dried hops had a costive effect when fed with the basal ration so that the amount of linseed cake had to be so fixed as to alleviate this slightly without causing undue looseness during feeding with the basal ration alone, so that the basal ration consisted finally of 500 gm. meadow hay chaff and 120 gm. of linseed cake meal per head per day. Fed on the experimental ration, four days were sufficient to accustom the sheep to the ration, and at the end of the seventh day the animals



were normal and in good spirits. As soon as the animals were brought on to the basal ration looseness of faeces set in, but this gradually passed, and on the seventh day of this period the animals were again in a normal state. Sheep III developed some looseness during the sampling period, at which time sampling was stopped for four days, after which samples were again taken, the animal being kept on the ration for a corresponding period after the other two. During the whole of the experimental periods the animals were in good health and spirits and consumed their rations eagerly. Water was given *ad lib.*, but no account was taken of the exact volumes consumed.

#### *Experimental periods.*

The following rations were given during the experimental periods:

*Period 1.* 12 days, 500 gm. meadow hay chaff, 120 gm. linseed cake meal and 100 gm. dried spent hops per head per day.

*Period 2.* 12 days, basal ration of 500 gm. meadow hay chaff and 120 gm. linseed cake meal.

#### *Sampling and analysis of feeding stuffs.*

The meadow hay chaff was obtained in large quantity and thoroughly mixed before being rebagged. The linseed cake was thoroughly ground and well mixed. During the actual feeding period samples of each food was set by in well-corked bottles when each ration was made out, each sample for analysis being a composite one for four days' feeding. The agreement in the analyses obtained later from such a large number of composite samples was satisfactory.

#### *Sampling and analysis of excreta.*

During the sampling periods, 4-day composite samples of faeces were collected. The fresh faeces were weighed and finely ground each day and an aliquot portion set away in a stoppered bottle until four days' samples had been collected. Crude protein was determined in this sample and loss of moisture to give air-dry faeces determined. The analysis of the air-dry samples were proceeded with almost immediately.

The urines were measured each day and the whole samples kept under toluene and well mixed before analysis. They were analysed as soon as time permitted.

**Table I. *Digestibility of basal ration.* (Period 2.)**

Average daily ration: 500 gm. hay chaff and 120 gm. linseed cake meal.

	Total dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	Nitrogen- free extractives gm.	Crude fibre gm.	Ash gm.
Consumed:							
Meadow hay	5301.0	5012.0	376.8	95.4	2328.8	1920.0	289.0
Linseed cake	1299.0	1157.3	428.7	128.7	417.0	110.6	72.3
Total	6600.0	6169.3	805.5	224.1	2745.8	2030.6	361.3
Voided:							
Sheep I	2534.0	2265.4	300.2	92.0	1111.8	761.4	268.6
Sheep II	2521.0	2258.4	278.1	90.3	1131.6	753.4	262.6
Sheep III	2592.0	2336.0	290.4	87.5	1141.7	816.4	256.0
Digested:							
Sheep I	4066.0	3903.9	505.3	132.1	1634.0	1269.2	92.7
Sheep II	4079.0	3910.9	527.4	133.8	1609.2	1277.2	98.7
Sheep III	4008.0	3833.3	515.1	136.6	1604.1	1214.2	105.3
Digestion coefficients:							
Sheep I	61.60	63.28	62.73	58.95	59.51	62.50	25.65
Sheep II	61.81	63.40	65.46	59.71	58.59	62.88	27.32
Sheep III	60.74	62.13	63.95	60.95	58.41	59.78	29.14
Mean	61.38	62.94	64.05	59.87	58.84	61.72	27.37

The results obtained for this period (Period 2) were satisfactory, there being good agreement between the digestion coefficients for the three sheep.

**Table II. *Basal ration plus dried spent hops.* (Period 1.)**

	Total dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	Nitrogen- free extractives gm.	Crude fibre gm.	Ash gm.
Consumed:							
Basal ration as per Table I.							
Dried spent hops	1130.0	1053.7	258.2	100.0	397.1	300.4	74.3
Voided:							
Sheep I	3484.0	3123.9	498.7	139.3	1441.0	1044.9	360.1
Sheep II	3436.0	3087.8	476.9	134.5	1437.5	1038.2	348.9
Sheep III	3545.0	3188.3	491.5	134.6	1465.3	1096.9	356.7
Voided from basal ration:							
Sheep I	2534.0	2265.4	300.2	92.0	1111.8	761.4	268.6
Sheep II	2521.0	2258.4	278.1	90.3	1136.6	753.4	262.6
Sheep III	2592.0	2336.0	290.4	87.5	1141.7	816.4	256.0
From spent hops:							
Sheep I	950.0	858.5	198.5	47.3	329.2	283.5	91.5
Sheep II	915.0	828.7	198.8	44.2	300.9	284.8	86.3
Sheep III	953.0	852.3	201.1	47.1	323.6	280.5	100.7
Digested:							
Sheep I	180.0	197.2	59.7	52.7	67.9	16.9	—
Sheep II	215.0	227.0	59.4	55.8	96.2	15.6	—
Sheep III	177.0	203.4	57.1	52.9	73.5	19.9	—
Digestion coefficients of dried spent hops:							
Sheep I	15.93	18.67	23.13	52.70	17.10	5.63	—
Sheep II	19.02	21.50	23.01	55.80	24.23	5.19	—
Sheep III	15.66	19.26	22.12	52.90	18.51	6.63	—
Mean digestion coefficients	16.87	19.81	22.75	53.80	19.95	5.82	—

The agreement between the results for the three sheep during this period was not so good as for the basal ration period, but was still satisfactory considering the small amount and nature of the added food. The greatest discordance appeared in the digestibility of the nitrogen-free extractives, but it must be understood that these values are affected cumulatively by disagreements in the coefficients for the other nutritive constituents. There was close agreement between the digestible coefficients for the crude protein, but, as was expected for a coarse fodder of this class, there was some discordance between the coefficients for the ether extract and crude fibre.

The low digestibility of all the constituents other than the ether extract was brought to view during this period, and this decreased considerably the digestibility of the dry matter of the ration. A little over a half of the ether extract only was digested. The spent hops depressed considerably the digestibility of the mineral constituents of the basal ration, since, in the faeces of Period 1, a greater amount of undigested mineral matter appeared than could be accounted for by the mineral matter added in the spent hops.

Utilising the digestible coefficients found in this period (2) it was possible to calculate the percentage of digestible nutrients in the dried spent hops:

	Percentage	Percentage of dry matter
Digestible crude protein ...	4.90	5.20
Digestible ether extract ...	4.48	4.76
Digestible N-free extractives	6.60	7.01
Digestible crude fibre ...	1.46	1.55

Production starch equivalent (Kellner) per 100 lb. dried spent hops = 24.5.

Production starch equivalent (Kellner) per 100 lb. dry matter = 26.01.

It can be seen (Table III) that except for Sheep III in the basal ration period all sheep showed a slight negative nitrogen balance. The sheep gained in weight slightly during the spent hops period, but, except for Sheep III, remained at constant weight during the basal ration period.

There was slight loss of mineral matter (Table IV) during the first period, but the three animals showed positive retention during the basal ration period. This partly explains the anomalous results obtained for the digestibilities of the mineral constituents, but does not give a quantitative explanation of the lowering of mineral digestibility by the addition of spent hops to the basal ration.

Table III. *Nitrogen balance during trial and sheep weights.*

Average daily nitrogen balances					
Period 1.	Nitrogen consumed	Nitrogen voided			Nitrogen retained (+)
		In faeces	In urino	Total	
Basal and hops:	gm.	gm.	gm.	gm.	gm.
Sheep I	14.18	6.65	8.45	15.10	- 0.92
Sheep II	14.18	6.66	8.79	15.45	- 1.27
Sheep III	14.18	6.66	7.98	14.64	- 0.46
Period 2.					
Basal:					
Sheep I	10.74	4.08	7.93	12.01	- 1.27
Sheep II	10.74	3.71	8.07	11.78	- 1.04
Sheep III	10.74	3.88	6.53	10.41	+ 0.33
Sheep weights					
		Sheep I lb.	Sheep II lb.	Sheep III lb.	
Period 1	{ July 3rd	105	98	95	
	{ July 16th	107	100	97	
Period 2	{ July 30th	106	101	100	

Table IV. *Mineral balance.*

Average daily mineral balances					
Period 1:	Consumed	Voided			Mineral matter retained (+)
		In faeces	In urine	Total	
	gm.	gm.	gm.	gm.	gm.
Sheep I	36.30	30.01	7.32	37.33	- 1.03
Sheep II	36.30	29.75	7.01	36.76	- 0.46
Sheep III	36.30	29.73	6.84	36.57	- 0.27
Period 2:					
Sheep I	30.11	22.39	7.14	29.53	+ 0.58
Sheep II	30.11	21.88	7.33	29.21	+ 0.90
Sheep III	30.11	21.33	7.85	29.18	+ 0.93

*The ether extract.*

The dried spent hops contained 8.33 per cent. ether extract which, being high for this class of foodstuff, called for further examination, and with this end in view a large quantity was prepared from the dried material by Soxhlet extraction. The last traces of ether were removed by keeping over strong sulphuric acid in a desiccator, owing to the decomposition of some of the oil at 100° and the driving off of some of the residual essential oils still left in the hops. The extract was isolated as a viscous brown oil which on keeping partly crystallised out, the whole liquefying at 22.5° on warming. Constants for the oil were determined according to the usual procedure for coloured fats, the results appearing in Table V.

Table V. *Constants of the "oil" from dried spent hops.*

Melting point ... ..	22.5°
Solidifying point (partial) ...	16.5°
Saponification value (mg. KOH)	193.4
Reichert-meissel value ... ..	10.3
Iodine value (Hubl) ... ..	28.4
Percentage insoluble fatty acids and unsaponifiable matter ...	96.7
Unsaponifiable matter ... ..	2.7
Acid value ... ..	17.2 to 28.3 (variable)

The oil contained an appreciable amount of volatile fatty acids and was broken up fairly readily on heating at 100° as was deduced from the variable acid values of the samples of extracts obtained. The iodine value was low. The oil was not completely soluble in hot alcohol (90 per cent.), showing that there was still a fair quantity of "hop oil" left in the spent hops, but the oil dissolved fairly easily in warm alcoholic potash. The oil smelled strongly of hop oil. The high percentage of unsaponifiable matter was as expected from a fibrous food of this nature.

#### SUMMARY.

Dried spent hops possess a high absorptive capacity, and attention is drawn to its use as a "filler" to absorb such by-products as molasses and treacle.

The material possesses a high crude protein value and its ether extract is very high for a fibrous food, while the amount of crude fibre is the same as in good meadow hay. There is a high percentage of mineral matter present.

The digestibility of the material has been determined by feeding with hay chaff and linseed cake meal to three sheep. The spent hops were not readily eaten and could only be included in a ration in an amount equal to one-seventh of the dry weight of the total ration.

Its digestibility is low, a fifth of the crude protein and the nitrogen-free extractives, one-half of the ether extract, one-twentieth of the crude fibre and one-fifth of the total organic matter only being digestible. The production starch equivalent was 24.5.

Spent hops included in the ration had the property of depressing the digestibility of the mineral constituents of the basal ration. The ether extract was examined in detail and was found to contain residual hop oil. The total extract was of moderate volatile fatty acid content, high saponification value compared with vegetable fat, low iodine value, and

contained an appreciable amount of unsaponifiable matter. The oil broke up readily under the effects of heat, and samples obtained had variable acid values.

The author is indebted to Profs. H. A. D. Neville and S. Pennington for the kind interest they took in the work, to Messrs Thorley of King's Cross, London, for supplying the consignment of spent hops, and to the Brewers' Society, a grant from which defrayed the expenses of the investigation.

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# OILINESS IN MILK.

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## A PRELIMINARY NOTE.

DURING recent years a large number of samples of milk having a peculiar flavour have been sent to this Institute by farmers, retailers, and wholesalers of milk.

The flavour is marked and has almost invariably been described as "oily." In many instances it has been referred to as the "castor oil flavour."

Although, as would be expected, the opinions of individuals on the flavour of such samples were not unanimous the majority agreed that "oily" was an appropriate name and since this is the name by which this taint is known to the trade it is proposed to retain it.

## OCCURRENCE.

"Oiliness" must be a very widespread taint, since samples have been received from all parts of England and some from Scotland. The quantity of milk represented by the samples received amounted to a very large total, and since it is certain that many more outbreaks must have occurred than were reported to this laboratory, the loss to the industry must be very great.

A peculiar feature of this taint is that it has not been known to occur during the summer, the complaints always being received in the late autumn, continuing through the winter and ending with the cold weather in the spring. The maximum number of complaints were received during spells of colder weather. Two cases occurring in the summer were in milk which had been kept in a cold store.

## DEVELOPMENT.

In the course of these investigations it has been noted that "oiliness" invariably requires time for development, with the result that milk which left farms in a perfectly sweet condition was found on arrival at the distributors' premises some hours (14 to 24) later, to be so oily as to necessitate its return to the producer. In other instances milk which arrived in good condition at a depot was found to have developed "oiliness"

after it had been pasteurised and retained for similar periods before distribution. Such milk is quite useless for sale as milk, and its value for butter making is extremely doubtful.

For a long time all the samples of milk received in the laboratory contained comparatively large numbers of bacteria, and in view of the fact that a time factor was involved and that in some cases the taint could be transferred from a tainted sample to milk which was quite normal, a search was made for bacteria which it was at first assumed were the cause of the taint.

Against the bacteriological theory was of course the fact that the temperatures, at which the taint developed, were comparatively low. However, as bacteria which develop at temperatures such as are encountered during winter in England are known, it was thought that the search for micro-organisms was justified.

Such organisms were in fact isolated, but none could, within the usual "incubation" period, be shown to cause a taste exactly corresponding to that described as "oily."

It was therefore assumed that a combination of organisms in "working association" must be involved, and much work was done on this assumption.

Here again no success was achieved and no great progress towards a solution of the problem was made until several samples of milk, containing very few organisms of any kind were received from producers.

Further light was thrown upon the subject when a visit was paid to a farm of 150 cows. A long search failed to reveal any abnormality amongst the animals in the herd, but it was found that the cooler was old and dirty and on hygienic grounds the farmer was advised to replace it. This was done and the taint which had persisted at intervals for weeks, at once disappeared.

It was obvious, therefore, that the cooler was a factor in the problem.

Washings with sterile saline were taken from the cooler, and bacteriological examinations made. These revealed large numbers of organisms of several varieties. None of these, however, when inoculated collectively or separately into milk could be shown to be capable of causing oiliness. It seemed, therefore, that a bacteriological explanation of "oiliness" was untenable.

It had, however, been noticed that some of the tinning had disappeared from the surface of the cooler, which in common with all farm coolers, was made of tinned copper. A small area of the copper was exposed and milk in passing over the surface of the cooler came in contact with it.



A cooler in a similar condition was set up in the laboratory and fresh milk passed over the surface. A portion, untreated, was kept as a control. After being kept at 50° F. for 16 hours it was found that the milk which had passed over the surface of the experimental cooler was markedly oily although immediately after cooling it had been perfectly normal in taste. Further, bacteriological examination showed that no increase had taken place in the very small numbers (a few hundreds per cubic centimetre) of bacteria present in the milk when cooled.

This experiment was repeated many times with milk from various sources and on every occasion "oiliness" could be induced as long as the temperature of storage remained comparatively low. When, however, temperatures approximating to those ruling during the summer in this country were tried, "oiliness" did not make its appearance.

It was finally ascertained that "oiliness" was the result of a reaction catalysed by copper which was, however, present in very minute quantities. These quantities were found to be far too small to be estimated by chemical means, but other methods of estimation are being tried.

On some farm coolers, examined in the course of tracing the source of "oiliness," the area of copper exposed was so small as to render it doubtful whether anyone already alive to the capabilities of copper in inducing "oiliness" would have considered it sufficient to cause the trouble.

It was further found that oxygen is essential to the reaction and that no "oiliness" could be induced in milk containing traces of copper and kept in an atmosphere of nitrogen.

In spite of the fact that no specific bacteria have been shown to cause "oiliness," experiments have led to the view that they are concerned in the limitation of the production of "oiliness" to cold weather. Either they deprive the catalyst (copper) of its oxygen which they use in their metabolism, or, by virtue of the fact that at summer temperatures their growth is so active as to change the pH of the milk to such an extent that the action of the copper does not proceed. These points are being investigated.

More than twenty years ago Golding and Feilmann drew attention to the fact that copper has a marked influence in the spoilage of milk and considered that the phenomenon was in some way associated with bacterial growth. Similar conclusions have been reached by American workers, much of whose work has appeared in journals not generally circulated here.

In previous studies, however, the action of copper on milk has usually

been investigated by the addition of copper salts in varying quantities to the milk and their influence on bacterial growth studied. The writer's experiments point to the fact that amounts of copper capable of retarding the growth of bacteria are sufficient to give milk a definite metallic flavour, which is not in the least like the "oiliness" which very minute quantities of this metal can induce. Experiments have shown that these amounts of copper are probably quite incapable of influencing the growth of bacteria in the absence of any other factor.

This research has, up to the present, shown that,

(1) Oiliness is due to the catalytic oxidising action of exceedingly minute amounts of copper which are taken up by milk at various points in its handling. Oiliness is not identical with other flavours induced by larger, but still very small, amounts of the metal.

(2) The reaction is, to a large extent at any rate, dependent on free access to oxygen in the molecular state.

(3) The reaction is probably limited by rises in temperature which correspond to summer conditions.

(4) Bacteria, although not directly involved in the reaction, act in a retarding capacity either by their own absorption of oxygen or by the production of acidity which carries the system outside the limiting  $pH$  or both.

(5) It is possible that the small amounts of metal may exercise an effect on bacterial growth in combination with temperature.

No details are given here because the research is not yet complete, but it was felt that "oiliness" in milk is of such practical importance and the action of such very minute amounts of copper in fresh milk so little recognised that this preliminary note should be published.

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# BULK IN ANIMAL FEEDING.

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(With One Plate and Two Text-figures.)

FROM the time of the earliest researches in animal feeding there has always been an endeavour to define the limits of capacity of an animal for food in one way or another; Thaer, for example, in his work with cattle, before the extensive use of concentrates, considered a ration in terms of hay or its equivalent. It was fifty years later, when great advances had been made in chemistry, that foods began to be considered in terms of their constituents, and Grouven appears to have been the first to state the capacity for food of milking cows according to the dry matter content of the ration.

Later Wolff and Lehmann accepted the method of associating the live weights of animals with certain requirements of dry matter, and since then most investigators of animal nutrition have adopted the method, which is one of very great convenience. It does not follow that the different investigators have been in agreement as to the method of stating the dry matter requirement, or as to the actual standards desirable. While most workers have held that the object to be aimed at was to feed dry matter according to the live weight of the animal, some have allowed much more latitude for the standards than others. Armsby<sup>(1)</sup> and Kellner<sup>(2)</sup> have considered that one was feeding correctly if the dry matter supplied was within 20 per cent. and 10 per cent. of the standards respectively; the former has also suggested that the requirement for dry matter needs determining for each individual animal.

Recently Boutflour<sup>(3)</sup> has stated that one cause of inferior milk yields in milch cows is due to too bulky a ration, and he gives for an average dairy cow, weighing 11 cwt., a ration containing a *maximum* of 33 lb. dry matter; unfortunately, Boutflour does not give the results of any adequately controlled experiments in support of his contention. Leroy<sup>(4)</sup> has described a method of controlling the bulk of a ration by calculating "coefficients of bulk"; the coefficients are calculated by dividing the

kilograms of dry matter of a ration by its content of fodder units, and he takes the coefficient of grass (1.3) as the working basis for ruminants. Each species of animal is given its own range of coefficients.

It seemed to the writers that the practice of stating the capacity of an animal for food in terms of dry matter may be misleading, since there is no evidence that with different foods the same weights of dry matter will occupy identical bulks "in the stomach," even when attention is confined to concentrates alone. The writers therefore adopted a new line of investigation in which efforts were made to obtain measurements of the actual bulk which foods would occupy in the stomach.

Preliminary work in the laboratory indicated very considerable variations in the swelling of foods after soaking, and feeding experiments have given evidence that such swelling may have a marked influence on the amount of food consumed by young pigs. The results of the work, which is necessarily of a preliminary nature only, are detailed in this paper.

#### A NEW POINT OF VIEW.

The object of the investigation was to measure the bulk occupied by equal weights of different foods *in the stomach*. In the experimental work, the writers fell back on the simple method of determining the increase in volume of foods when soaked in water at body temperature, a method which might be expected to give sufficiently suggestive data, and which, from the point of view of possible future routine measurements, gives an easily reproducible technique. It is realised that this method is open to criticism from several points of view, but it was considered that the results obtained would be sufficiently indicative of differences in bulk after soaking to give valuable results.

The exact technique employed was as follows: Each food was milled and sieved, and only that portion passing through sieve 30 was used for the experiments. Ten grams were weighed out and placed in a clean dry cylinder. The cylinder was gently tapped until the volume occupied by the foodstuff remained constant. This figure gave the dry volume of the food, *i.e.* the bulk occupied by the *dry* food. To the cylinder was now added sufficient water at 38° C. to bring the volume to 50 c.c. (in the case of high-swelling foods, 100 c.c.), the whole being well mixed with a long stirring-rod; this enabled every feeding stuff to take up the maximum amount of water it was capable of absorbing. The cylinder was placed in an incubator at 38° C., and readings of the volume occupied by the wet food taken at intervals of two, four and six hours. As a check, in order

to eliminate errors due to individual differences in the settling of the food, the whole contents of the cylinder were then transferred to a large centrifuge tube, and the tube centrifuged at 3000 R.P.M. for five minutes. Portions of the solid portion were removed and the percentage of moisture in it determined: this figure could be checked against that obtained by calculation from the wet volume of that quantity of food equivalent to 10 gm. of its dry matter, since in the case of palm kernel cake for instance, although the dry volume of the milled cake was 24 c.c., the actual volume of the cake equivalent to 10 gm. of dry matter was just 10 c.c.—the other 14 c.c. being air spaces between the particles. This was demonstrated repeatedly by measuring the volume of water necessary to bring the total volume to 50 c.c. of a foodstuff equivalent to 10 gm. of its dry matter; invariably 40 c.c. of water were required and not 50 c.c. less the dry volume. For calculation, therefore, the method is as follows:

Volume of P.K.C. (equivalent to 10 gm. dry matter) after soaking	= 45 c.c.
Actual volume of P.K.C.	= 10 c.c.
Volume of water in 45 c.c. of wetted food	= 35 c.c.
Percentage of water = $\frac{35}{45} \times 100$	= 77.8
Figure obtained practically by evaporation	= 76.5 %

The figures obtained in this way showed excellent agreement with the results obtained practically. The results obtained with a series of foodstuffs are shown in Table I and Table II, where it will be seen that those foodstuffs which the farmer describes as heavy appear at the head of the list with the smallest final volumes. When two or more determinations for the same feeding stuff are given in Table I the samples are of different origin.

The following points were brought out in these experiments:

(1) *Rate of swelling.* The foodstuffs undergo maximum swelling in a very short time, the increase in bulk occurring immediately on wetting, although measurements cannot be made until the food has settled to the bottom of the cylinder. This is important from the point of view of swelling in the stomach, the experiments indicating that the food will have swelled to its full extent immediately it reaches the stomach.

(2) *Amount of swelling.* The amount of swelling varies enormously, being lowest in the meat and fish meals, and highest in some vegetable cakes (linseed and coconut cakes). Calculated to 10 gm. of dry matter, the variation in volume after soaking extends from 24 c.c. to 88 c.c. (See Pl. II, fig. 1.)

(3) *Percentage of moisture.* The percentage of moisture in the wetted foods has been determined in two ways; first, by calculation from the increase in volume on wetting, and second, by direct determinations on

Table I. *Swelling of milled foodstuffs on soaking.*

Food	Volume in c.c. occupied by the equivalent of 10 gm. dry matter		Percentage of moisture		Percentage increase of swelling on soaking
	Dry	Wet	Calculated	Determined	
Ext. whale meal	22.0	24.0	58.5	66.0	9.1
Unext. whale meal	17.5	26.0	61.5	57.5	48.5
White fish meal	16.0	31.5	68.0	67.0	97.0
Rice meal	23.0	32.0	69.0	66.5	39.2
French maize gluten feed	20.5	32.0	69.0	71.0	56.2
	24.5	33.3	—	—	36.0
	25.5	36.0	—	—	41.2
Maize meal	18.0	32.5	69.5	64.5	81.0
Barley meal	23.0	37.0	73.0	58.0	61.0
Wheat sharps	21.5	37.0	73.0	68.5	72.3
Bean meal	19.5	37.5	73.5	63.5	92.5
Dec. cottonseed cake	16.0	39.0	74.0	66.5	144.0
Ext. soya meal	16.0	40.5	75.5	76.5	153.0
Undec. cottonseed cake	23.5	42.5	76.5	—	81.0
	28.0	42.5	76.5	71.5	52.0
	37.0	54.0	—	—	46.5
Garton's maize gluten feed	36.0	65.0	—	—	80.6
	20.0	42.5	76.5	78.5	112.0
	19.5	42.5	76.5	79.0	118.0
Unext. soya cake	17.0	43.0	77.0	75.0	153.0
Dec. ground nut meal	17.5	43.5	77.0	73.5	148.0
	18.0	45.0	—	—	150.0
	16.5	47.5	—	—	187.9
Palm kernel cake	24.0	45.0	78.0	76.5	87.5
	22.0	42.0	—	—	90.9
	18.5	45.5	78.5	73.5	146.0
Ext. palm kernel meal	46.5	52.5	81.0	82.0	12.9
	35.0	47.0	—	—	34.3
	32.0	57.0	82.5	79.5	78.0
Brewer's grains	27.5	88.0	88.5	88.0	220.0
Coconut cake	25.5	88.0	88.5	87.0	245.0
Linseed cake	21.0	76.0	—	—	261.9
Alfalfa meal*	37.5	98.0	—	—	—

\* Results unsatisfactory owing to bad settling.

Table II. *Swelling of foodstuffs (as fed) on soaking.*

Food	Volume in c.c. occupied by the equivalent of 10 gm. dry matter		Percentage in- crease of swelling on soaking
	Dry	Wet	
Mixture A	22.0	50.0	127.3
Mixture B	22.0	34.0	70.0
Mixture C	23.0	51.5	124.0
Mixture D	22.0	31.0	41.0
Mixture X	14.0	34.0	143.0
Mixture Y	17.0	25.0	47.0
Calf mixture (ground)	18.5	38.5	108.0
Balanced ration (ground)	21.0	35.0	66.0

the solid residue after centrifuging. The results shown in Table I demonstrate the quite good correlation between the calculated and determined values. The interesting and significant results in this table are those obtained with the high-swelling foods, such as linseed and coconut cakes: it will be seen that the percentage of moisture in these foods after wetting is of the same order as the percentage of moisture in such bulky foods as roots and green fodder; *i.e.* that in so far as the feeding of bulk is concerned, a small weight of these cakes is as bulky in the stomach as a large weight of roots or green fodder.

(4) *Effect of Pressure.* While the method used in the determinations of swelling is simple and straightforward, criticism might be made that it does not take into account possible differences in pressure, either in the settling of the food in the cylinders, or in the wider application of the effect of pressure of the stomach walls after ingestion. In order to meet

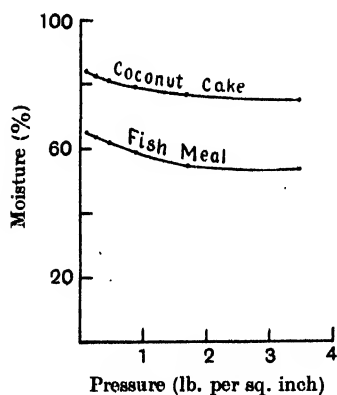


Fig. 1.

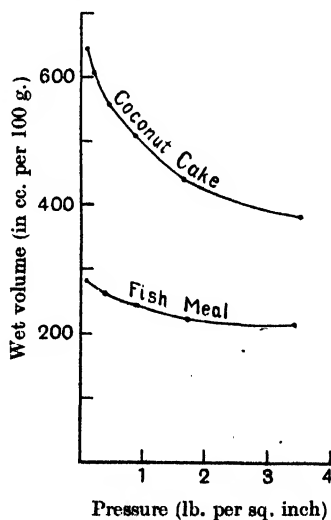


Fig. 2.

this criticism, experiments have been carried out in which the pressure on the wetted foods was varied, and the final volumes and percentages of moisture in the resulting product determined. The method employed was to soak the food thoroughly, and then to transfer the mass to a small cheese-mould, in which it could be subjected to various pressures by means of suitable weights. The results of experiments carried out on coconut cake and on fish meal (as representing extremes in swelling) are shown in the accompanying graphs. (See also Pl. II, fig. 2.)

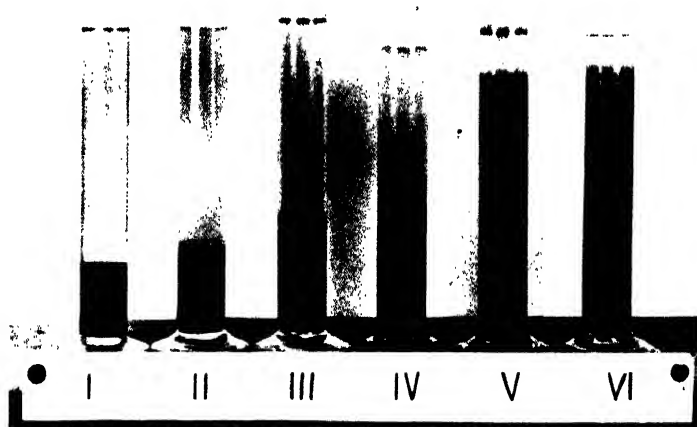


Fig. 1. Method of measuring the swelling of foods.

The foods illustrated are: I. Ext. whale meal; II. Fish meal; III. French maize gluten feed; IV. Dec. groundnut meal; V. Coconut cake; VI. Linseed cake.



Fig. 2. Relative bulk occupied by wetted foods after pressure of 4 lb. per sq. in.  
(Left, Fish meal; right, Coconut cake.)





The results show that, although with high pressures there are absolute differences due to variation in pressure, there are no significant relative differences in either the degree of swelling or the percentage of moisture. It appears, therefore, that criticism from this point of view is invalid.

(5) *Factors causing variation in swelling.* The swelling of food might be due to:

- (a) imbibition of water by proteins,
- (b) the swelling of starch granules, and/or
- (c) the influence of the salts present in the food.

Probably all these factors are contributory, though it is not possible directly to correlate swelling with any one of them. There appears, however, to be a definite correlation between the swelling and the percentage of protein, if one excepts the foods in which the proteins have been denatured during manufacturing processes, *e.g.* meat and fish meals and extracted products. The possible action of individual constituents of the carbohydrates (for instance, the pentosans) should not be omitted. The fact that mixtures of foods (for instance, equal parts of coconut cake and fish meal) give results which agree with the proportions used, suggests that salts have a very small effect, if any. Further work on the chemical side is necessary to clear up these points.

### CAN BULK LIMIT FOOD CONSUMPTION?

The laboratory work having shown that some feeding stuffs swell little on moistening while others swell much, and that with some foods a given quantity on soaking will give a small final volume while with others a similar quantity will give a large final volume, it was decided to carry out feeding trials with pigs and calves to determine whether the volume occupied on entering the stomach would be a limiting factor in the consumption of food.

Feeding trials were conducted with pigs from 40 to 80 lb. live weight, and with calves mostly of 2 to 3 cwt. live weight, in order to test this point. As the young animals were continually growing in size and weight, and as their food requirements were also increasing, it was decided to employ the "change-over" system, to be described in the succeeding section dealing with the pig feeding trials.

### FEEDING TRIALS WITH PIGS.

In the experimental work with pigs, four rations were fed, two of which (rations A and C) had high final volumes and two of which

(rations *B* and *D*) had low final volumes on soaking. These rations were as follows:

High-swelling foods		Low-swelling foods	
Ration <i>A</i>		Ration <i>B</i>	
2 parts ground coconut cake.		2 parts French maize gluten feed.	
1 part ext. dec. groundnut meal.		1 part ext. whale meal.	
2 parts sharps.		2 parts sharps.	
2 parts barley meal.		2 parts barley meal.	
Starch equivalent, 71.0 per cent.		Starch equivalent, 69.0 per cent.	
Digestible protein, 15.4 per cent.		Digestible protein, 15.0 per cent.	
Ratio of volumes of equal weights of <i>A</i> : <i>B</i> , after soaking, 138.4 : 100.00.			
Ration <i>C</i>		Ration <i>D</i>	
1 part ground coconut cake.		1 part French maize gluten feed.	
2 parts sharps.		2 parts sharps.	
5 per cent. fish meal.		3 per cent. fish meal.	
Starch equivalent, 71.2 per cent.		Starch equivalent, 70.0 per cent.	
Digestible protein, 15.6 per cent.		Digestible protein, 16.3 per cent.	
Ratio of volumes of equal weights of <i>C</i> : <i>D</i> , after soaking, 162.0 : 100.00.			

It will be seen that the rations were fairly equally balanced as regards starch equivalent and digestible protein, but that the volumes to which equal weights of the mixtures swelled on soaking were widely dissimilar.

In all, fourteen pigs were used; eight in comparing rations *A* and *B*, and six in comparing rations *C* and *D*. The method adopted was the "change-over" method; for this method to be successful it was necessary that the experiment should be completed in as short a time as possible, consistent with accuracy, because of the rapid growth of the pigs.

In actual practice the technique worked well, and it was possible to compare the weights of the different rations eaten with considerable accuracy.

The time-table of the feeding is given below:

	1st week	2nd week	3rd week	4th week	5th week	6th week
First group: Pigs 1, 2, 3, 4	Change to ration <i>A</i>	Ration <i>A</i>	Change to ration <i>B</i>	Ration <i>B</i>	Change to ration <i>A</i>	Ration <i>A</i>
Second group: Pigs 5, 6, 7, 8	Change to ration <i>B</i>	Ration <i>B</i>	Change to ration <i>A</i>	Ration <i>A</i>	Change to ration <i>B</i>	Ration <i>B</i>
Third group: Pigs 9, 10, 11	Change to ration <i>C</i>	Ration <i>C</i>	Change to ration <i>D</i>	Ration <i>D</i>	Change to ration <i>C</i>	Ration <i>C</i>
Fourth group: Pigs 12, 13, 14	Change to ration <i>D</i>	Ration <i>D</i>	Change to ration <i>C</i>	Ration <i>C</i>	Change to ration <i>D</i>	Ration <i>D</i>

Prior to the experimental period the pigs had been fed on a weaning mixture of 95 per cent. toppings and 5 per cent. fish meal. The weights of the pigs at the beginning of the experimental period are shown in Table III.

In practice the method of feeding adopted was as follows: The pigs were fed individually. The ration for each pig was weighed out, and to this was added, for high-swelling mixtures, four times the weight of

water, and for low-swelling mixtures, twice the weight of water. These quantities had been shown to give in each case suitable pastes of uniform consistency. After the pigs had been fed it was an easy matter to collect

Table III.

No. of pig	Sex	Weight lb.	No. of pig	Sex	Weight lb.
1	♀	43	5	♀	30
2	♂	40	6	♂	47
3	♂	36	7	♂	38
4	♂	31	8	♂	35
9	♀	43	12	♀	45
10	♂	43	13	♂	50
11	♂	22	14	♂	53

and weigh the residue. It was, however, always the endeavour to feed to each pig only what it would eat, so that a large excess of food, which might tend to put it off its feed, might be avoided. This object was achieved.

The results of the pig-feeding experiments may be looked at from two points of view, *i.e.* increase in live weight, and amounts of mixtures consumed.

#### INCREASE IN LIVE WEIGHT.

It is considered that the feeding periods were too short for any reliable figures to be obtained on the gains of live weight from each food mixture, but although the figures in the following table should be accepted with caution, they do show that in three of the groups greater live weight increases were obtained each week with low-swelling foods than with high-swelling foods.

Table IV. *Live Weight Increases.*

Group	Mean live weight increases in lb. for periods of trial on high-swelling foods	Mean live weight increases in lb. for periods of trial on low-swelling foods
1	7.95	8.20
2	7.30	8.80
3	6.80	9.55
4	10.00	9.30
Mean	<u>8.01</u>	<u>8.96</u>

#### AMOUNTS OF MIXTURES CONSUMED.

The weights of food eaten during each experimental period are given in Table V, together with the averages for each group. The weights of meal eaten during the change-over periods are not given, since they are of no experimental significance.

Table V. *Weights of food consumed by pigs.* (In lb. per day.)

Pig No.	High-swelling foods			Low-swelling food
	1st period	3rd period	Mean	2nd period
1	2.09 $\pm$ 0.11	3.14 $\pm$ 0.08	2.61	2.49 $\pm$ 0.16
2	2.32 $\pm$ 0.11	2.74 $\pm$ 0.12	2.53	3.38 $\pm$ 0.17
3	2.00 $\pm$ 0.13	2.24 $\pm$ 0.15	2.12	2.96 $\pm$ 0.15
4	2.06 $\pm$ 0.11	2.33 $\pm$ 0.15	2.20	2.82 $\pm$ 0.19
Mean	2.12	2.61	2.36	2.91
9	2.44 $\pm$ 0.05	2.24 $\pm$ 0.24	2.34	3.21 $\pm$ 0.20
10	2.50 $\pm$ 0.00	2.92 $\pm$ 0.05	2.71	3.86 $\pm$ 0.18
11	1.84 $\pm$ 0.09	2.29 $\pm$ 0.13	2.07	2.62 $\pm$ 0.18
Mean	2.26	2.48	2.37	3.23

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Pig. No.	Low-swelling foods			High-swelling food
	1st period	3rd period	Mean	2nd period
5	2.53 $\pm$ 0.13	2.90 $\pm$ 0.21	2.72	2.69 $\pm$ 0.12
6	2.90 $\pm$ 0.12	3.42 $\pm$ 0.21	3.16	3.24 $\pm$ 0.16
7	2.50 $\pm$ 0.21	3.49 $\pm$ 0.19	3.00	2.67 $\pm$ 0.09
8	2.51 $\pm$ 0.24	3.05 $\pm$ 0.17	2.78	2.92 $\pm$ 0.05
Mean	2.61	3.21	2.91	2.88
12	2.88 $\pm$ 0.15	4.05 $\pm$ 0.27	3.47	3.24 $\pm$ 0.06
13	3.32 $\pm$ 0.18	4.78 $\pm$ 0.12	4.05	3.76 $\pm$ 0.09
14	3.43 $\pm$ 0.12	4.62 $\pm$ 0.22	4.03	3.94 $\pm$ 0.05
Mean	3.21	4.48	3.85	3.64

When the upper half of Table V is studied it is seen that, with the single exception of Pig No. 1, a small quantity of food was eaten when the food mixture was capable of swelling to a high final volume; moreover, the differences of food consumed are definitely significant, and in this case the high swelling of the food did limit the quantity eaten.

It would, however, be interesting to know whether the feeding of a high-swelling mixture would tend to make a more capacious stomach, and so prepare the way for the consumption of a comparatively large quantity of the low-swelling mixture. This is a debatable point, and one on which we have no definite information.

When the lower half of the table is considered, *i.e.* when the foods consumed were in the order of low-high-low swelling, the results are not so definite and consistent.

It may be noted, however, that, with the exceptions of Pigs No. 6 and No. 8, a heavier weight of the low-swelling food mixture was eaten daily by all the pigs. It may be stated therefore that the experiments with pigs indicate that with high-swelling foods the volume to which the foods swell on soaking may be a limiting factor in the ingestion of food.

The factors concerned are by no means simple, and further extended feeding trials are required before any final conclusion can be reached.

NOTE. In considering the above results, it is important to avoid confusion of thought as regards the limiting volume of the stomach in feeding. The above results indicate that with a high-swelling mixture the limited capacity of the stomach prevents sufficient bulk of food from being eaten, and therefore may not allow the pig sufficient nutrients for its maximum growth and maintenance. This does not necessarily imply that the amount of food taken by an animal is controlled by the capacity of the stomach, *i.e.* that an animal will always eat until its stomach is full. The amount of food may be controlled rather by the requirements of nutrients than by the emptiness of the stomach. This point is illustrated by the figures in Table VI, where the calculated *volumes* of each mixture eaten by the pigs are given. It will be seen that in every case the volume of low-swelling food eaten is very much less than the volume of high-swelling food (which may be taken to provide figures of the actual food capacity of the stomach), indicating that, though the appetite of the pig is satisfied with the low-swelling food, the stomach itself is by no means full.

Table VI. *Final (wet) volumes of foods consumed by pigs\*.*

Pig No.	High-swelling foods			Low-swelling food	Pig No.	Low-swelling foods			High-swelling food
	1st period c.c.	3rd period c.c.	Mean c.c.			1st period c.c.	3rd period c.c.	Mean c.c.	
1	4180	6280	5230	3598	5	3656	4190	3923	5380
2	4640	5480	5060	4884	6	4190	4943	4567	6480
3	4000	4480	4240	4277	7	3612	5043	4328	5340
4	4120	4660	4390	4075	8	3627	4407	4017	5840
Mean	4235	5225	4730	4205	Mean	3771	4638	4205	5760
9	5302	4868	5085	4301	12	3859	5427	4643	7040
10	5432	6345	5888	5172	13	4449	6405	5427	8170
11	3998	4976	4397	3511	14	4596	6191	5394	8562
Mean	4911	5389	5150	4328	Mean	4301	6003	5152	7910

\* One pound of the mixtures occupy the following volumes after soaking:  
A, 2000 c.c.; B, 1445 c.c.; C, 2173 c.c.; D, 1340 c.c.

#### FEEDING TRIALS WITH CALVES.

The experimental work with the calves was more difficult than with pigs. Nine of the twelve foods experimented with were single foods only, while the remaining three were mixtures. In some cases much difficulty was encountered in getting the calves to eat the single foods owing to the

lack of palatability, consequently a small proportion of "balanced ration" was added to get the calves to eat the foods with more zest. The composition of the "balanced ration," which was very palatable, was as follows:

Dec. groundnut meal (extracted)	2 parts
Soya meal (extracted)	2 "
Rice meal	5 "
Palm kernel cake	2 "
Maize gluten feed	2 "
Pollards	2 "
Molasses	2 "

With some other calves it was impossible to get them to eat the desired quantities of the foods.

The method of feeding was the "change-over" system. Each calf was given a certain weight of the selected food each morning; later hay was fed *ad libitum*, but in each case the meal and the hay eaten were weighed. Individual feeding was practised, and the calves were allowed to drink all the water they wished, but on each Tuesday, Wednesday and Thursday of the experimental period the water was weighed. It was considered that the larger the final volume of the meal fed the smaller would be the weight of hay eaten if the bulk of the meal had any effect on the food consumption, and vice versa. Obviously, then, a positive effect would be measured by the inverse weight of hay eaten, thus involving considerable calculation.

#### FEEDING STUFFS USED IN THE EXPERIMENT.

Table VII (p. 403) gives the foods used in the experiment with data of the foods and calves; animals 96 and 108 were Guernsey heifers whereas the remaining calves were shorthorns.

The proportions by weight of the constituents of mixtures X and Y and the calf mixture are as follows:

X	Y	C.M.
1 part ground linseed cake	1 part extracted palm kernel meal	3 parts linseed cake
1 part ground coconut cake	1 part French maize gluten feed	3 parts kositos No. 5
1 part dec. groundnut meal	1 part ext. whale meal	3 parts crushed oats
2 parts white maize meal.	2 parts white maize meal.	1 part fish meal.

Table VIII gives a summary of the results obtained in the calf feeding experiments, and Table X gives the calculated volumes of the foods tabulated in Table VIII.

A study of Tables VIII and X shows that feeding of meals of low and high volumes after soaking had no consistent effect on the appetite as

measured by the inverse quantity of hay eaten, and on the whole any results lie within the range of the probable error. It seems desirable, however, that the experiment should be repeated with extreme swelling feeding stuffs making use of the knowledge gained in this work. However, it seems that foods of extreme swelling power must be used if any positive result is to be obtained, otherwise any effects would be masked by the high percentage probable error of the amount of hay eaten.

Table VII.

Name of foods	Percentage increase of swelling of food as fed	Herd Nos. of calves used	Age of animal at commencement of expt. days	Wt. of calf at commencement of expt. lb.
1. (a) Coconut cake	220	96	96-567	599
(b) Ground barley	61	108	108-505	628
2. (a) *B.R. and bran (1 : 5)	54	116	116-201	294
(b) B.R. and French maize gluten feed (1 : 5)	47	117	117-188	252
3. (a) Mixture X	143	—	—	—
(b) Mixture Y	47	119	119-186	263
(c) French maize gluten feed	43	120	120-177	267
(d) B.R. and French maize gluten feed (1 : 8)	46	—	—	—
4. (a) Dec. groundnut meal	162	—	—	—
(b) Calf mixture	108	121	121-144	229
(c) †C.M. and dec. groundnut meal (1 : 5)	153	124	124-101	200
5. (a) B.R. and palm kernel cake (1 : 5)	85	118	118-193	299
(b) B.R. and palm kernel meal (1 : 5)	31	122	122-152	262

\* B.R. represents balanced ration.

† C.M. represents calf mixture.

It may be that the ruminant animal is well able to accommodate itself to considerable changes of volume of the total food eaten, and the structure and anatomy of the capacious stomachs lend weight to this hypothesis.

#### WATER CONSUMPTION.

Table IX gives the average daily water consumption of the calves during the three stages of the experiment; it has been indicated previously that the weights of water are based on the actual quantity drunk on three consecutive days in the middle of each week, and not on the weight consumed on every day of the experimental period. It will be seen that the individual factor is the most prominent, since some calves drank much water during all stages of the experiment, while others drank



Table VIII. *Weights of food and hay eaten. Large-small-large changes of final volumes of foods.*

<i>First feeding period.</i>		Length of feeding period in days	Average daily weight of meal eaten—in lb.	Average daily weight of hay eaten—in lb.	Length of change over period in days
No. of Calf	Concentrate used				
96	Coconut cake	7	4.0 $\pm$ 0	10.21 $\pm$ 0.68	7
116	B.R. + bran (1:5)	7	3.0 $\pm$ 0	9.40 $\pm$ 0.61	7
118	B.R. + P.K.M. (1:5)	6	2.66 $\pm$ 0.21	8.12 $\pm$ 0.82	6
119	Mixture X	6	2.0 $\pm$ 0	8.25 $\pm$ 0.93	8
Average			2.91	9.00	
<i>Second feeding period.</i>					
96	Barley meal	8	4.0 $\pm$ 0	10.31 $\pm$ 0.56	8
116	B.R. + French M.G.F. (1:5)	16	3.0 $\pm$ 0	11.00 $\pm$ 0.64	6
118	B.R. + P.K.C. (1:5)	6	2.87 $\pm$ 0.13	8.54 $\pm$ 0.71	5
119	French M.G.F.	6	1.87 $\pm$ 0.19	8.04 $\pm$ 0.97	8
Average			2.94	9.47	
<i>Third feeding period.</i>					
96	Coconut cake	7	4.0 $\pm$ 0	12.00 $\pm$ 0.88	
116	B.R. bran (1:5)	7	3.0 $\pm$ 0	10.14 $\pm$ 0.67	
118	B.R. + P.K.M. (1:5)	7	2.67 $\pm$ 0.17	9.86 $\pm$ 1.06	
119	Mixture x	6	2.0 $\pm$ 0	9.67 $\pm$ 0.65	
Average			2.92	10.42	
<i>Small-large-small changes of final volumes of foods.</i>					
<i>First feeding period.</i>					
108	Barley meal	7	4.0 $\pm$ 0	10.21 $\pm$ 0.68	7
117	B.R. + French M.G.F. (1:5)	7	3.0 $\pm$ 0	8.80 $\pm$ 0.53	7
119	French M.G.F.	6	1.87 $\pm$ 0.19	8.04 $\pm$ 0.97	9
120	Mixture Y	11	3.0 $\pm$ 0	7.32 $\pm$ 0.64	
121	Dec. G.N.M.	9	3.0 $\pm$ 0	6.64 $\pm$ 0.65	11
122	B.R. + P.K.C. (1:5)	6	2.58 $\pm$ 0.16	7.87 $\pm$ 0.84	6
124	Calf mixture	6	2.67 $\pm$ 0.19	3.92 $\pm$ 0.88	10
Average			2.87	7.83	
<i>Second feeding period.</i>					
108	Coconut cake	8	4.0 $\pm$ 0	10.06 $\pm$ 0.37	8
117	B.R. + bran (1:5)	10	3.0 $\pm$ 0	8.88 $\pm$ 0.41	7
119	Mixture X	6	2.0 $\pm$ 0	9.92 $\pm$ 0.71	13
120	Mixture X	8	3.0 $\pm$ 0	7.00 $\pm$ 0.64	9
121	Calf mixture	8	3.0 $\pm$ 0	5.40 $\pm$ 1.12	9
122	B.R. + P.K.C. (1:5)	5	2.85 $\pm$ 0.13	7.25 $\pm$ 1.86	6
124	C.M. + Dec. G.N.M. (1:5)	8	3.0 $\pm$ 0	5.81 $\pm$ 0.57	11
Average			2.98	7.76	
<i>Third feeding period.</i>					
108	Barley meal	7	4.0 $\pm$ 0	12.07 $\pm$ 0.50	
117	B.R. + French M.G.F. (1:5)	7	2.68 $\pm$ 0.30	9.54 $\pm$ 0.67	
119	B.R. + French M.G.F. (1:8)	6	*2.12 $\pm$ 0.13	11.16 $\pm$ 0.24	
120	Mixture X	11	2.86 $\pm$ 0.17	7.36 $\pm$ 1.64	
121	Dec. G.N.M.	7	2.89 $\pm$ 0.01	7.25 $\pm$ 1.29	
122	B.R. + P.K.C. (1:5)	6	2.62 $\pm$ 0.08	8.79 $\pm$ 1.24	
124	Calf mixture	6	2.42 $\pm$ 0.08	6.33 $\pm$ 0.79	
Average			2.80	8.93	

\* 3 lb. daily, except on three occasions.

Table IX.

*Water consumption of calves.**Large-small-large changes of final volumes.*

No. of Calf	Period 1		Period 2		Period 3	
	Food eaten	Daily wt. of water (lb.)	Food eaten	Daily wt. of water (lb.)	Food eaten	Daily wt. of water (lb.)
96	Coconut cake	43.0 $\pm$ 3.0	Barley meal	45.0 $\pm$ 5.6	Coconut cake	50.0 $\pm$ 2.2
116	B.R. + bran	38.3 $\pm$ 5.0	B.R. + French M.G.F.	42.0 $\pm$ 1.3	B.R. + bran	40.0 $\pm$ 2.0
118	B.R. + P.K.M.	47.3 $\pm$ 1.9	B.R. + P.K.C.	48.5 $\pm$ 0.3	B.R. + P.K.M.	53.0 $\pm$ 1.5
119	Mixture X	33.7 $\pm$ 1.8	French M.G.F.	42.0 $\pm$ 1.4	Mixture X	44.3 $\pm$ 0.9
	Average	40.6		44.4		46.8

*Small-large-small changes of final volumes.*

108	Barley meal	25.0 $\pm$ 0.7	Coconut cake	27.0 $\pm$ 5.8	Barley meal	26.0 $\pm$ 1.1
117	B.R. + French M.G.F.	34.8 $\pm$ 3.2	B.R. + bran	39.3 $\pm$ 2.3	B.R. + French M.G.F.	40.5 $\pm$ 2.1
119	French M.G.F.	42.0 $\pm$ 1.4	Mixture X	44.3 $\pm$ 0.9	B.R. + French M.G.F.	37.0 $\pm$ 1.7
120	Mixture Y	38.5 $\pm$ 6.4	Mixture X	38.8 $\pm$ 4.8	Mixture Y	33.9 $\pm$ 8.4
121	Dec. G.N.M.	41.5 $\pm$ 0.7	C.M.	42.6 $\pm$ 0.9	Dec. G.N.M.	48.7 $\pm$ 2.2
122	B.R. + P.K.C.	29.7 $\pm$ 1.4	B.R. + P.K.M.	26.0 $\pm$ 0.3	B.R. + P.K.C.	28.2 $\pm$ 0.9
124	C.M.	27.3 $\pm$ 0.7	C.M. + Dec. (i.N.M.)	34.3 $\pm$ 1.2	C.M.	37.8 $\pm$ 1.8
	Average	34.1		36.0		36.0

B.R. = Balanced ration.

P.K.M. = Palm kernel meal.

M.G.F. = Maize gluten feed.

C.M. = Calf mixture.

G.N.M. = Groundnut meal.

P.K.C. = Palm kernel cake.

Table X. *Calculated volumes of food and water consumed in c.c.**Large-small-large changes of final volumes.*

No. of Calf	Period 1			Period 2			Period 3		
	Vol. of meal fed	Vol. of hay fed	Vol. of hay, meal and water	Vol. of meal fed	Vol. of hay fed	Vol. of hay, meal and water	Vol. of meal fed	Vol. of hay fed	Vol. of hay, meal and water
96	13,568	25,525	58,682	5,925	25,775	52,152	13,568	30,000	66,296
116	6,468	23,500	47,375	3,660	27,500	50,249	6,468	25,353	49,998
118	4,561	20,300	46,359	3,044	21,350	46,437	4,578	24,650	53,316
119	2,916	20,625	38,858	2,139	20,100	41,328	2,916	24,175	47,225
Av.	6,878	22,488	47,818	3,692	23,681	47,541	6,883	26,044	54,208

*Small-large-small changes of final volumes.*

108	5,925	25,525	42,812	13,568	25,150	50,989	5,925	30,175	47,917
117	3,660	22,000	41,477	6,468	22,200	46,530	3,270	23,850	44,527
119	2,139	20,100	41,328	2,916	24,800	47,850	2,425	27,900	47,141
120	3,352	18,300	39,150	4,374	17,500	39,509	3,195	18,400	37,003
121	4,716	16,000	40,178	5,117	13,500	37,979	4,543	18,125	44,802
122	4,200	19,675	37,374	4,779	18,125	34,721	4,265	21,975	39,057
124	4,554	9,800	26,762	4,716	14,525	34,820	4,128	15,825	37,133
Av.	4,078	18,857	38,440	5,991	19,400	41,771	3,964	22,321	42,511

The volume of 1 lb. of hay after soaking was found to be 2500 c.c.

The volumes of the feeding stuffs are calculated after soaking.

relatively little. The type of food eaten does not appear to have affected the water consumption, and the addition of the volume of water drunk to the total volumes of food eaten as stated in Table X does not appear to throw any light on the problem.

The authors willingly acknowledge the assistance given by Mr A. Rowlands, B.Sc., in carrying out the pig feeding trials.

#### SUMMARY.

1. As a result of soaking in water at body temperature (38° C.), foods show variations in swelling ranging from approximately 10 per cent. to 260 per cent.

2. The volumes of equal weights of different foods after soaking also vary within wide limits. The percentages of moisture in the soaked foods show corresponding variations, being in some cases as high as those found in roots or green fodder.

3. These facts have led the authors to put forward a new conception of "bulk" in assessing the value of a ration.

4. Feeding experiments have been carried out to determine how far this factor of bulk is applicable in practice.

5. In the case of pigs of 40 to 80 lb. live weight, the bulk occupied by the foods *did* affect the quantity of food taken.

6. With calves, the swelling capacity of the concentrated food did not yield any definite results so far as food consumption was concerned.

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# SOME FACTORS AFFECTING THE EVAPORATION OF WATER FROM SOIL. II.

## THE DISCONTINUITY OF THE DRYING PROCESS.

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In a previous series of papers (2), (3), (4), (5) the writer published the results of a study of the rate of drying of a number of colloidal materials including wool fabric, soils, ball clay, kaolin, sand, silt, etc. and showed that with certain exceptions, to be mentioned later, when the first differentials of change of moisture content with time of drying,  $-\frac{dw}{dt}$ , were plotted against moisture contents, the curves obtained were of the type shown in Fig. 1, which is the actual curve obtained for the rate of drying of a wool fabric. The three straight portions were accurately expressed by the equations:

$$(1) \quad -\frac{dw}{dt} = k_1.$$

$$(2) \quad -\frac{dw}{dt} = k_2 w; \text{ or } -\frac{dw}{dt} = A + k_2 w,$$

when as was the case with sand, silt, etc., the curve would not cut the origin on extrapolation but would cut off an intercept,  $A$ , from the vertical axis.

$$(3) \quad -\frac{dw}{dt} = k_3 (w - c),$$

$c$  being an intercept cut off by extrapolation from the horizontal axis.

Below 5 per cent. water content for wool or 2 to 3 per cent. for clay the rate curves bent round to the origin, drying becoming very slow in this region. With some materials, *e.g.* sand, silt, kaolin, this curved portion did not exist; the third straight portion of the rate curve passed to the origin,  $c$  in equation (3) becoming equal to 0.

With clay soil the middle linear portion of the drying curve was in part, or entirely, curved, suggesting that some additional factor or factors were involved in the evaporation.

Later these conclusions were reviewed and in part controverted by Keen, Crowther and Coutts<sup>(9)</sup> in an elaborate and critical examination of the experimental technique. They showed that, with the wet materials they

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worked with, viz. Rothamsted soil, kaolin and silt, a considerable variety of drying curves could be obtained for the same material as a consequence of small changes in environmental conditions. Even with apparently constant conditions they were frequently unable to get even approximate agreement between replicate experiments. These experimental results certainly justify the authors' contention that "caution must be exercised at present in associating precise physical explanations with the shape of the complete rate curves" and that "the question of formulating an explanation for the whole course of the rate curves needs further careful consideration." This is true; and some of the conclusions arrived at by the present writer on the basis of his earlier work on drying completely wet soils cannot be sustained in the light of the work of Keen, Crowther and Coutts. In particular, the rate curve obtained by the writer<sup>(3)</sup> for the "fractionated" Rothamsted subsoil cannot now be regarded as giving a typical picture of the course of the drying process for this material; an entirely different type of curve would probably have been obtained by slight changes of technique and the suggested explanations of the curvature must be abandoned.

The disagreement between the replicate curves of Keen *et al.* both as regards position and shape is striking, the more so in view of the excellent agreement obtained by the writer in the case of wet woollen fabrics. With pieces of wool fabrics  $3\frac{1}{2}'' \times 2\frac{1}{2}''$  the following replicates were carried out:

Single thickness	...	...	...	...	...	6
One dried single thickness re-wetted and drying repeated						1
Two thicknesses sewn together	...	...	...	...	...	4
One double thickness re-wetted	...	...	...	...	...	1
Three thicknesses sewn together	...	...	...	...	...	4
One treble thickness re-wetted	...	...	...	...	...	1
						<hr/> 17

After making the obvious corrections for differing dry weight of the various thicknesses and for the extra surface presented by the edges of the additional layers, the experimental points fell with sufficient accuracy on the same smooth experimental curve so that the same rate curve applied to all the 17 virtual replicates. In this connection it is important to note that the materials used by Keen, Crowther and Coutts and by the writer in earlier papers *were all thoroughly wetted* at the commencement of the experiments. The point is important and fundamental. The difference

<sup>1</sup> Fisher (3), p. 673.

between the results is undoubtedly connected with the ease of movement of water (or water vapour) from point to point within the drying mass. The movement is extremely slow and irregular in the case of a thoroughly wetted compact colloidal mass such as clay or soil; it is comparatively easy and quick within a woollen fabric. In the latter case inequalities in concentration of moisture in the mass as drying proceeds, caused by the edges drying more rapidly than the central portion, are rapidly equalised by diffusion of moisture by capillarity (or by diffusion of vapour) from one part of the wool to another. It would appear that linear rate curves are obtained with materials like woollen fabrics in which the distribution of moisture is maintained constant, but not, as with wet clay or soil, when lack of adequate porosity prevents easy movement of internal moisture.

It is well known that there are two groups of factors that govern the evaporation of water from a mass of material in a drying chamber: (1) the water-solid system itself, and (2) the environmental conditions. Both these were discussed in the writer's earlier papers; the former he called internal factors, the latter external or drying conditions. Keen, Crowther and Coutts also discuss them and maintain that their experiments "show that the latter group exercises a controlling influence over the greater portion of the evaporation period." In this group of environmental conditions the authors include (a) simple diffusion of water vapour through the air to the acid, (b) bulk air movements due *e.g.* to (i) temperature gradients between acid and cover plates, (ii) temperature lowering of the drying mass itself due to evaporation, (iii) lower density of moist air, (iv) inevitable disturbances introduced by the weighings or by movement of the apparatus, (v) the geometry of the system.

In this paper it is desired to discuss these factors in relation to the general drying process. In the light of fresh experimental work by the writer covering a wide range of materials it is perhaps possible to build up a more definite and complete picture of the drying process than was possible from the earlier work of Keen, of the present writer, and of Keen, Crowther and Coutts.

Of the external factors listed above it is probable that simple diffusion of water vapour through the air to the acid plays an insignificant part in the drying process. It is known that a stationary film of air exists at the surface of all solid and liquid bodies and that the film may vary in thickness with conditions<sup>(10), (11)</sup>; it may be as much as 4 or 5 mm. in thickness. Across this film the water vapour lost during drying must pass by diffusion only. In the case for example of a wet material (or a dish of water)

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in a drying, *i.e.*, an unsaturated, atmosphere, we have at the actual wet surface of the stock a vapour pressure,  $p_1$ , equal to that of saturated water vapour at the particular temperature, while in the atmosphere itself the vapour pressure is  $p_0$ ,  $p_0$  being less than  $p_1$ . We have therefore in the film a vapour pressure gradient of  $p_1 - p_0/l$ ,  $l$  being the thickness of the film and under the influence of this gradient water vapour diffuses from the surface of the material to the outer surface of the stationary air film, *from which point it is swept away by convection and mixed with the*

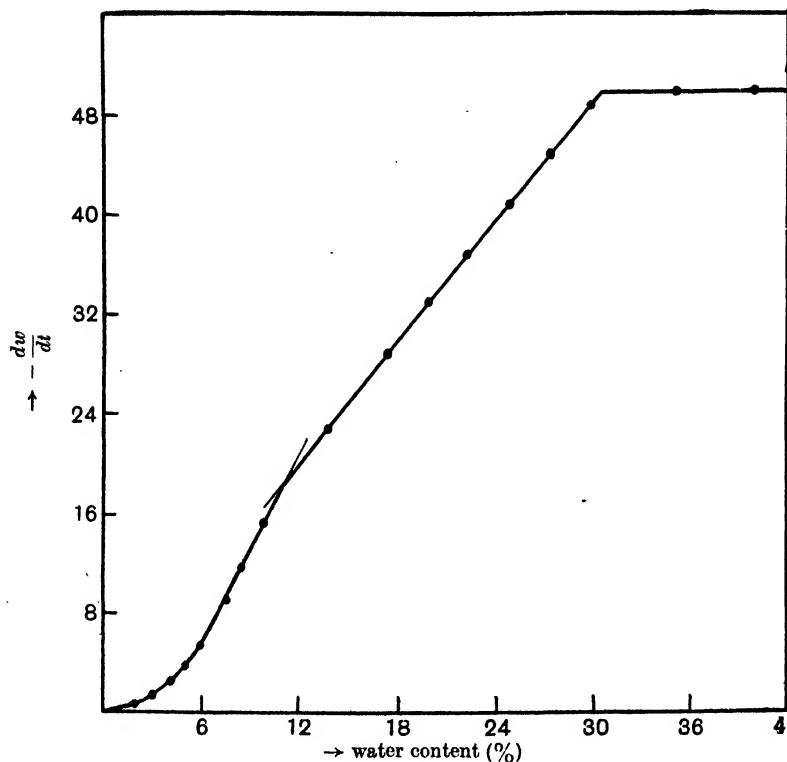


Fig. 1.

*main body of air and vapour*, or, under the experimental conditions dealt with here, is absorbed by the acid. These are the conditions obtaining in commercial driers (1), (12) (or in the natural drying of soils in the field) where the bulk movements of air are sufficiently obvious; under these conditions the rate curves obtained approximate to the linear type. It was with a view to carrying out a study of this process under rigidly defined conditions that the earlier work of the writer was done.

Under the experimental conditions employed by the writer these bulk movements or convection currents are less obvious but are undoubtedly operative and effective. The temperature gradients alone within the drying chamber (*a*) between the cover plates and the acid and (*b*) from the drying material outwards are adequate to produce movements of sufficient rapidity to cause mixing. As regards (*a*) one of Keen's experiments showed that a thermometer, the bulb of which was in the position normally occupied by the soil, showed a temperature  $3\frac{1}{2}^{\circ}\text{C}$ . below that of the acid or the thermostat; this would probably mean a gradient of 6 or  $7^{\circ}$  at least between acid and cover plates. As regards (*b*) much bigger gradients exist between the drying material and the surroundings as indicated by the results given in Table I. These data were obtained during a study of the evaporation of water from a small woollen glove fitted round the bulb of a thermometer. The thermometer was hung from the under side of the balance pan and the bulb was in the drying vessel in the position normally occupied by the soil<sup>1</sup>.

Table I.

Temperature of thermostats ° C.	Temperature of fabric	
	At start ° C.	At finish ° C.
25	9.8	21.5
35	14.0	29.5
45	17.8	37.5

The bulk air movements may be further increased by the inevitable movements of the stock due to the weighing and to the movements of the thermostat; this factor is however probably slight. Certainly the writer has never observed any effect on the smoothness of the curve provided the movements were not sufficient to cause internal disturbances in the drying material. This could not occur with wool fabrics; with air-dry powdery materials such as soils or wheat flours, starch, precipitated wheat proteins, etc., care has to be taken in weighing, and in moving the thermostat; any sudden jerk in the latter may disturb the close packing of the drying material and irregularities in the curve may result. One example of this is shown in Table II which contains the data for a sample of an ordinary blended wheat flour; the irregularity between  $6\frac{1}{2}$  and 5 per cent. *w* is plainly indicated and was probably caused by some unduly violent movement of the thermostat. It is interesting that after a while the drying process in this example settled down to a more normal course

<sup>1</sup> For a detailed account of this work see E. A. Fisher and G. Barker (8).



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and a linear rate curve was rejoined below 5 per cent.  $w$ . A similar and even more striking instance of departure from and subsequent return to a linear rate of drying is seen in Fig. 2, Curve B, which is for a very finely ground sample of Rothamsted subsoil.

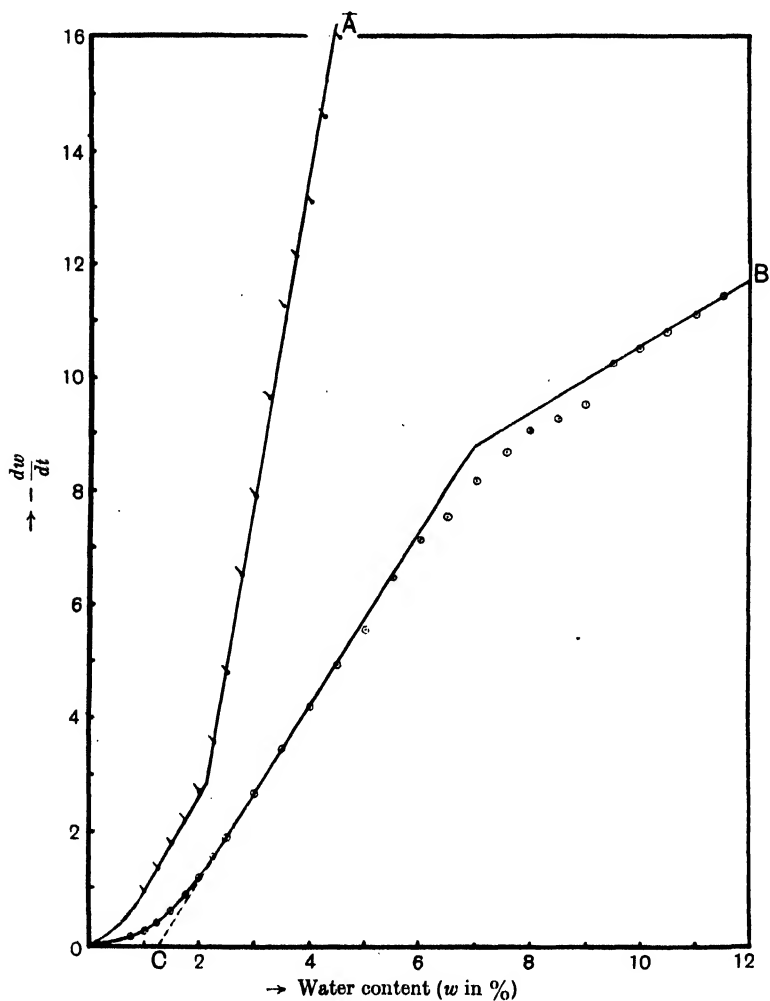


Fig. 2.

The lower density of moist air must be insignificant in its effect on bulk air movement in view of the magnitude of the temperature gradients.

It is difficult to see in what way the geometry of the system can come in as a serious factor affecting bulk air movements. It was possibly sug-

gested by Keen, Crowther and Coutts in view of the great differences in the shape of the rate curves when the soil was dried (a) in shallow pans, (b) on the under side of glass plates, and (c) in inverted weighing bottles. In the last case the rate curves approximated to the ideal limiting case of infinitely slow evaporation, *i.e.* to the vapour pressure curves. In this case however the bulk air movements were largely inoperative and what was being measured was the rate of diffusion of water vapour direct from the surface of the soil to that of the acid, aided and hastened by the lower

Table II. *Times of drying, observed and calculated, of a sample of flour.*

Water content (w) %	Time (observed) mins.	Time (calculated)* mins.
12.0	14.00	15.30
11.5	22.50	23.00
11.0	31.25	31.25
10.5	40.00	40.13
10.0	49.50	49.80
9.5	60.50	60.35
9.0	72.00	71.94
8.5	84.50	84.90
8.0	99.00	99.40
7.5	116.25	116.28
7.0	136.00	136.00
6.5	161.00	159.77
6.0	197.50	189.25
5.5	240.00	230.05
5.0	279.00	295.65
4.75	300.50	431.50
4.50	322.50	302.00†
4.25	349.00	322.50
4.00	374.00	347.75
3.75	406.25	373.50
3.50	437.50	405.75
3.25	477.00	438.00
3.00	524.00	476.50
2.80	573.00	521.25
		573.00

\* Calculated from the equation  $\log \frac{w_1 - c_1}{w_2 - c_1} = k_1 t$  in which  $k_1 = 0.003816$  and  $c_1 = 4.31$ .

† Experimental curve is slightly irregular here.

‡ Times below  $w = 4.75$  are calculated from the equation  $\log \frac{w_1 - c_2}{w_2 - c_2} = k_2 t$ , in which  $k_2 = 0.1092$  and  $c_2 = 1.90$ .

temperature of the soil which would produce some air movement in a very restricted space. *If the bulk air movements are adequate and the space not too restricted* the geometry of the system does not appear to be of great importance; thus linear rate curves have been obtained by the writer, and can be reproduced with considerable accuracy, from (a) wet horizontal pieces of wool fabric of varying shapes and sizes, evaporation taking place from both sides, (b) wet woollen gloves fitted to the thermometer bulb (evaporation from one side only, and that of a by no means

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simple geometrical shape), (c) from the *upper* surface of circular pans of *air dried* granular materials such as soils, wheat flour, starch, etc.

It is undoubtedly strongly suggested by experiment that the characteristic drying curve is of the linear and discontinuous type, and that, provided always the bulk air movements are adequate and the space not too restricted, external drying conditions are in themselves of less importance than the internal conditions of the drying system in determining the type of drying curve. It is essential that the movement of water or vapour through the drying mass be regular and uniform. This appears to be the case with powdery air-dry materials such as soil, clay, wheat flour, starch, etc., and with such materials linear and discontinuous rate curves are obtained. When the movement of water throughout the mass is slow and irregular, due to low porosity, as with *wet* clays, soils, dough, etc., the rate curves deviate from the normal linear type.

It seems probable that with air-dried granular materials moisture will move by capillarity from the interior to the surface of each separate compound particle and will there evaporate; the vapour will then diffuse through the air spaces to the surface of the mass and from there diffuse across the stationary air film as already described. As capillary connection between the compound particles is poor very little moisture interchange will occur between them except through the vapour phase; the whole mass acts, as it were, as a mulch; loss of moisture from it is comparatively slow but the rate curve obtained is essentially linear. With such materials a "steady state" is attained: at first there is uniform distribution of moisture throughout the mass; then as drying proceeds the periphery loses water faster than the centre and a moisture gradient is set up between centre and periphery; this moisture gradient must increase at first, reach a maximum and then decrease until finally at very low rates of drying and very low moisture contents the distribution must again become uniform. Such a "steady state," that cannot occur with wet or puddled clays or doughs of extremely low porosity, must be an important factor in determining the linear character of the drying curves. It is not unlikely that deviations from this character, such as those recorded by Keen, Crowther and Coutts and by the writer<sup>1</sup>, are determined more by lack of porosity of the drying material than by irregular air currents.

There is much experimental evidence to support this view. The fact that the rate of drying is faster at the periphery than at the centre of the material was demonstrated by the writer in the following way: a similar

<sup>1</sup> Fisher (3), p. 673.

experiment to that described above in connection with Table I was carried out at 35° C. with the following change in technique. A small hole was cut in the centre of a  $3\frac{1}{2}'' \times 2\frac{1}{2}''$  piece of the same fabric of which the glove was made. The hole was large enough to allow the thermometer bulb with its glove freedom of movement when hung through the hole. Both were then placed in position in the drying vessel and the rate of drying of the glove only was determined. Both with and without the surrounding flat fabric linear rate curves were obtained although the actual rates were very different in the two cases; thus

$$\begin{array}{ll} \text{without surrounding fabric} & K_1 = 143; K_2 = 4.47; \\ \text{with} & \text{,,} \quad \text{,,} \quad K_1 = 57.9; K_2 = 2.64. \end{array}$$

A sample of Rothamsted subsoil similar to that used in the earlier investigations was air dried, ground up in a mortar with a wooden pestle and as much as possible passed through a 5xx silk bolting cloth<sup>1</sup>. The moisture content was 7.36 per cent. The rate of drying was then determined in the usual manner. The tangents were calculated by the arithmetical method devised by Dr R. A. Fisher of Rothamsted<sup>2</sup>, and are shown in Fig. 2, Curve A. The curve is essentially linear and discontinuous in type although (a common feature of these curves) somewhat irregular at the "wet" end (not shown), *i.e.* prior to the attainment of a "steady state" by the soil. It is interesting to compare this curve with the one previously obtained with similar material thoroughly wetted. With the earlier curve the second linear portion suddenly changed direction into the third linear portion at 8 per cent. *w*, the third linear portion passing, apparently tangentially, into the curved portion which passed to the origin. With the air-dry sample an additional linear portion appears at 2 per cent. *w* which must at some point below 1 per cent. *w* curve round to the origin. This portion was missed in the earlier experiments owing to the small number of experimental points obtained at very low moisture contents. With the air-dry sample more material was employed and more frequent weighings taken over a longer period so that the curve could be followed as closely and as far as possible; 18 experimental points were obtained below 2 per cent. *w* as against 3 or 4 only in the earlier series. This additional linear portion is quite definite and characteristic of the drying curves, and really replaces a portion of the curved region of the earlier rate curves. It is very prominent in the rate curves for flour (see Fig. 4), starch and other cereal products, and in these cases extends from

<sup>1</sup> This had 68 threads per linear inch.

<sup>2</sup> For details of this method see E. A. Fisher (7).

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5-6 per cent.  $w$  down to 2-3 per cent. (7). It is not possible at present to explain this break, but it must have some significance; does it correspond to the point of maximum moisture gradient between centre and periphery of the drying mass suggested above?

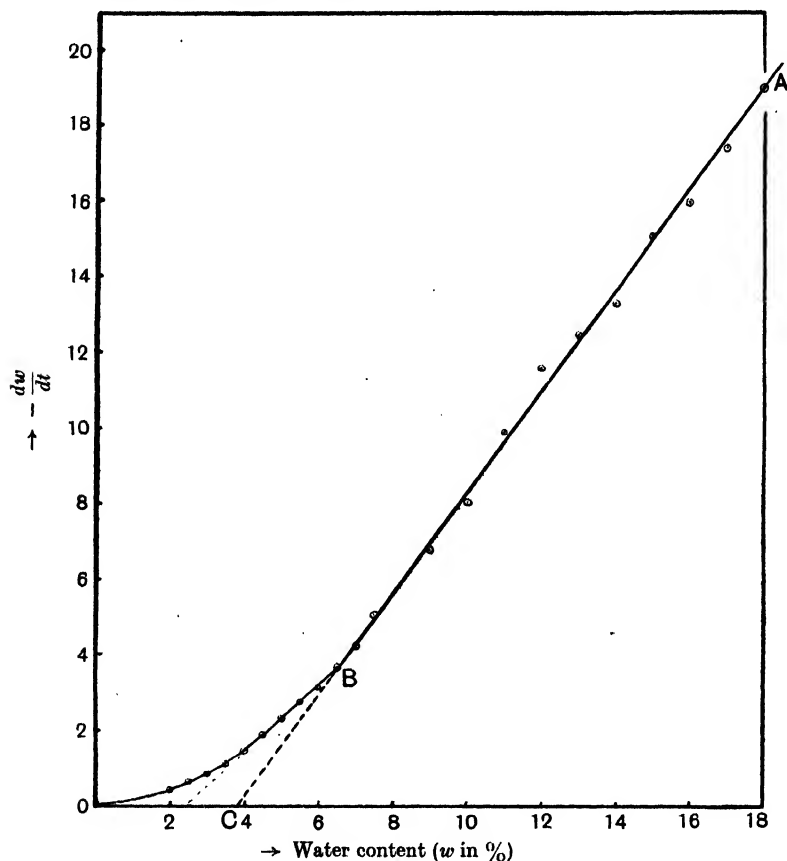


Fig. 3.

Another sample of the same subsoil and also one of a fine Dorset ball clay both of which had been ground to pass a 10xx silk bolting cloth<sup>1</sup> were kept in a vacuum desiccator for several weeks over 20 per cent. sulphuric acid. Each sample was thoroughly mixed with a spatula twice each week and again finally before drying in the apparatus. The rate curves were linear and discontinuous although somewhat irregular in places. The subsoil curve is shown in Fig. 2, Curve B; the irregularity

<sup>1</sup> = 113 threads per linear inch.

between  $6\frac{1}{2}$  and 9 per cent.  $w$  has already been referred to. The increased fineness of grinding has greatly reduced the rate of drying, although the dry weights were similar ( $A = 5.095$  gm.,  $B = 5.288$  gm.), and was also

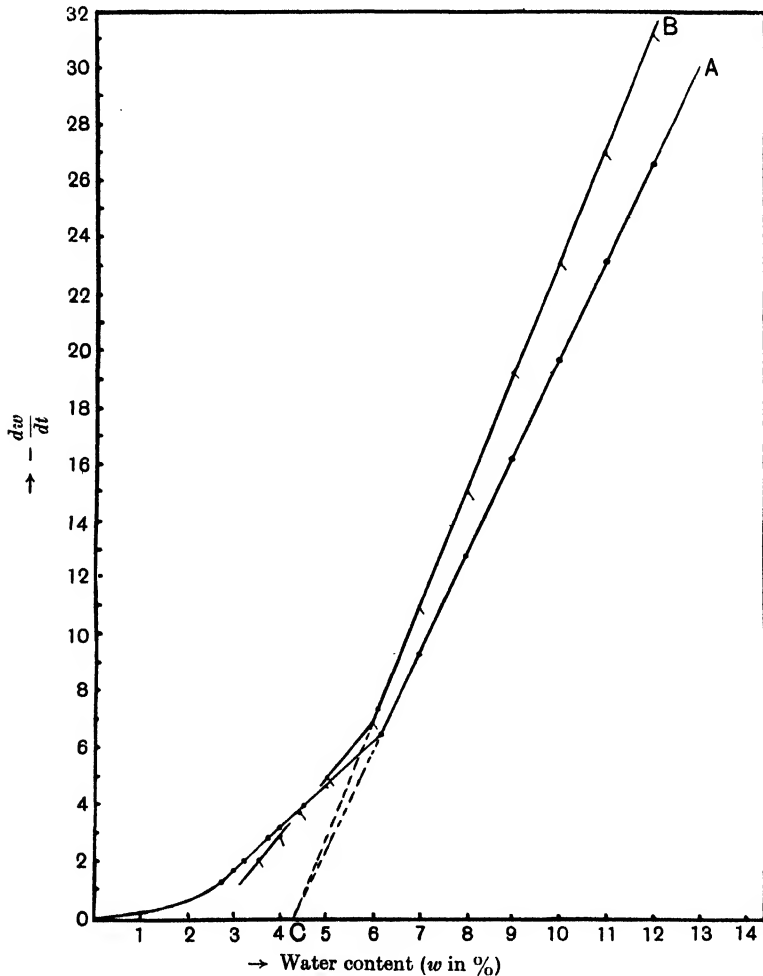


Fig. 4.

probably responsible for the observed irregularities; the powder showed no coherence and would be very sensitive to thermostat movements.

The curve for ball clay is given in Fig. 3; here again the curve is rather irregular but the linear character is unmistakable and the additional straight portion below  $6\frac{1}{2}$  per cent. is plainly visible.

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With flour, starch and precipitated wheat proteins the first sloping portions of replicate rate curves (when different dry weights are used) are not identical, but as shown in Fig. 4 on extrapolation converge to a point, *C*, on the horizontal axis. There is evidence that these intercepts are characteristic of the material<sup>1</sup>. Duplicate determinations have not been carried out with air-dried soil and clay, but it is possible that similar convergence of replicate curves would be found. If this were so the magnitude of the intercept, *OC*, might be of use in soil classification as considerable differences would be expected in the values of *OC* for different soils (cf. *OC* for soil and for ball clay shown in Figs. 2 and 3).

### SUMMARY.

Some of the factors affecting the rate of loss of water from a drying system are shortly reviewed. These factors fall into two groups: (1) the drying system itself, and (2) the environmental conditions. The second group may include (a) diffusion of water vapour through the air, (b) bulk air movements due to (i) temperature gradients between different parts of the drying vessel, (ii) temperature lowering of the drying mass itself due to evaporation, (iii) lower density of moist air, (iv) inevitable disturbances introduced by experimental conditions such as weighing or movement of apparatus, (v) the geometry of the system. It is shown that of the external factors the most important are (2 (a)), (2 (b) (i)) and (2 (b) (ii)); (2 (b) (iv)) may produce irregularities in the rate curves of air-dry granular materials; (2 (b) (iii)) and (2 (b) (v)) appear to have little or no effect.

The internal factors, *i.e.* the character of the drying system itself, appear to be of far greater importance than the environmental conditions in determining the type of drying curve. The generalisation is reached that the drying of any capillary system can be expressed by linear and discontinuous rate curves provided that the movement of moisture or of vapour through the drying mass is regular and uniform. When movement of moisture or vapour through the system is extremely slow and irregular, owing to low porosity, as is the case with wet clays, soils, doughs, etc., rate curves of various shapes may result and replication becomes impossible. There is evidence to show that the curvature exhibited by some of the earlier curves of the writer, and of Keen, and the later curves of Keen, Crowther and Coutts was due to such irregular moisture movements.

<sup>1</sup> For further details concerning this aspect of the problem see E. A. Fisher (7), *loc. cit.*

It is further shown that when different dry weights are employed in replicate experiments certain linear portions of the individual rate curves on extrapolation converge to a common point on the horizontal ( $w$ ) axis. The intercepts thus cut off may possibly be of more than passing interest; *e.g.* further investigation should show whether such intercepts can be employed as coefficients of granularity, whether they are affected by the colloidal content of the systems, or whether they can be used as, more or less, empirical constants in characterising a soil.

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# THE INFLUENCE OF THE AGE OF THE COW ON THE YIELD AND QUALITY OF THE MILK.

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(With One Text-figure.)

IN a former communication (1) a study has been made of the influence of the stage of lactation on the yield and quality of the milk, and in the following paper a similar investigation has been made of the influence of the age of the cow on the same factors.

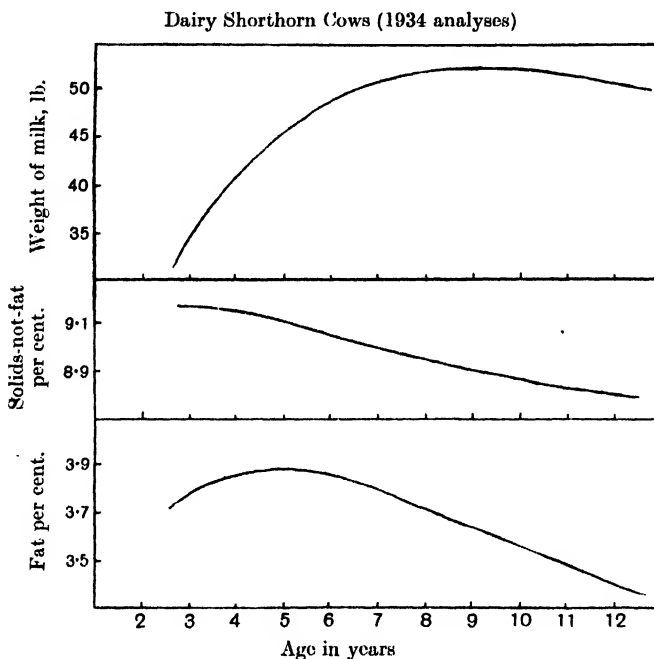
Mackintosh (2) finds a gradual increase in yield up to the 4th or 5th lactation period, and a decrease after the 7th or 8th; thus a heifer calving at  $2\frac{3}{4}$  to 3 years gives 60–70 per cent. of the amount it will produce as a mature cow; after the second calf it gives 80 per cent. and after the third 90 per cent. Tocher's results (3) show an increase from 85 per cent. of the maximum yield at 3 years to a maximum at  $7\frac{1}{2}$  years, and a subsequent fall to 90 per cent. at 12 years of age.

According to Mackintosh the quality of the milk is at its best after the first and second calves, the percentage of fat then decreasing slightly as the cow advances in age. Tocher finds a slight increase in the butter fat percentage as the cows increase in age between 2 and 5 years, and a gradual fall after 5 or 6 years. He also finds the regression of solids-not-fat on age to be linear, the percentage continuously decreasing with increasing age.

The conclusions of both Mackintosh and Tocher are based very largely on the analyses of the milks of Ayrshire cows, and no figures are given for other breeds. In this paper the effect of the age on the quality and quantity of the milk of nine different breeds of cows has been considered, the results used being those obtained at the Shows held by the British Dairy Farmers' Association during the past 48 years. The advantages of using these analyses have already been mentioned in a previous paper (1).

The disadvantage is that these results can hardly be regarded as absolute standards, since the show-yard conditions though identical for each breed are not those which prevail on the farm. However, whilst the position of the curves showing the relationship between the various factors and the age may, therefore, indicate too favourable a result with regard to the particular factor, it is concluded that the position of the curve with regard to the age axis is that which would be obtained in normal practice.

The cows were milked at 13 and 11 hour intervals, the longer interval being at night, and of the 6040 analytical results used in this paper, one



half were for the morning and the remainder for the evening milk. The results for the morning and evening milks were originally graphed separately, to show the change in the yield, percentages of fat and solids-not-fat with the age. It was found that exactly parallel curves were obtained, showing that age had no influence on the respective differences between the yield, percentages of fat and solids-not-fat in the morning and evening milks. Hence, for the purpose of comparison, the daily yield and daily percentages of fat and solids-not-fat have been considered for each breed in this communication.

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The stage of the lactation period has a great influence on both the yield and quality of milk, and since the results used in this paper are not the averages of complete lactation periods, but the analyses of single milkings only, this factor naturally influences and modifies the effects of the age of the cow.

It was found that the average number of days in milk differed for each breed, the limits being 41 days for Ayrshire, and 108 for Jersey, and thus this factor must be considered in making breed comparisons. The variation in the stage of lactation of the different ages of cow within the breeds, however, was not sufficiently large to mask the effect of the age, though there was a tendency in some breeds for it to become greater with the advance in age of the cows.

### RESULTS.

The effect of the age of Dairy Shorthorn Cows on the quantity and quality of their milk is illustrated in the figure.

The curves were drawn as nearly as possible through the average points, and the values of these curves are given in Table I. Tables II-IX give the values of similar curves obtained for the eight other breeds considered in the investigation. These tables also show the effect of age on the actual yield of fat and solids-not-fat, and the percentage of fat in the total solids. As in the former communication, where insufficient data have been available to make absolutely definite values possible, the probable figures are given in italics in the tables.

Table I. *Dairy Shorthorn Cows.*

Age (years)	2	3	4	5	6	7	8	9	10	11	12
Average No. of days since calving	48	47	41	41	41	52	56	60	62	95	65
No. of analyses	410	170	142	336	378	258	128	70	42	12	8
Yield (lb.)	31.0	37.9	43.5	47.5	50.2	51.5	52.2	52.2	52.0	51.7	51.0
Fat (%)	3.71	3.82	3.88	3.88	3.83	3.75	3.68	3.60	3.54	3.45	3.37
Solids-not-fat (%)	9.18	9.13	9.13	9.08	9.01	8.95	8.92	8.88	8.84	8.81	8.79
Fat (lb.)	1.15	1.45	1.69	1.84	1.92	1.93	1.92	1.88	1.84	1.78	1.72
Solids-not-fat (lb.)	2.84	3.48	3.97	4.31	4.52	4.61	4.65	4.63	4.60	4.55	4.48
Total solids (lb.)	3.99	4.93	5.66	6.15	6.44	6.54	6.57	6.51	6.44	6.33	6.20
Fat in total solids (%)	28.8	29.4	29.9	30.0	29.8	29.5	29.2	28.9	28.6	28.1	27.8

Number of analyses = 1954.

Average number of days since calving = 49 days (2-10 years).

Table II. *Red Poll Cows.*

Age (years)	2	3	4	5	6	7	8	9	10	11
Average No. of days since calving	54	58	65	64	88	75	85	86	77	91
No. of analyses	192	92	116	112	86	54	38	40	14	10
Yield (lb.)	30.9	36.5	39.8	41.7	42.8	43.6	43.9	44.0	43.9	43.6
Fat (%)	3.76	3.88	3.89	3.79	3.69	3.62	3.59	3.57	3.56	3.54
Solids-not-fat (%)	9.25	9.24	9.15	9.10	9.06	9.02	8.98	8.94	8.93	8.91
Fat (lb.)	1.16	1.41	1.55	1.58	1.58	1.58	1.58	1.57	1.56	1.54
Solids-not-fat (lb.)	2.86	3.47	3.64	3.80	3.88	3.93	3.94	3.93	3.92	3.88
Total solids (lb.)	4.02	4.88	5.19	5.38	5.46	5.51	5.52	5.50	5.48	5.42
Fat in total solids (%)	28.9	28.9	29.9	29.4	28.9	28.7	28.6	28.5	28.5	28.5

Number of analyses = 754.

Average number of days since calving = 72 days (2-9 years).

Table III. *Lincoln Red Shorthorn Cows.*

Age (years)	2	3	4	5	6	7	8	9	10	11
Average No. of days since calving	41	42	38	34	64	70	52	74	53	110
No. of analyses	78	66	38	50	42	36	28	20	6	6
Yield (lb.)	32.6	38.5	43.7	47.7	50.3	51.5	52.0	52.0	51.7	50.8
Fat (%)	3.57	3.69	3.74	3.73	3.68	3.62	3.55	3.49	3.42	3.34
Solids-not-fat (%)	9.22	9.20	9.14	9.08	9.02	8.96	8.90	8.85	8.80	8.7
Fat (lb.)	1.16	1.42	1.63	1.78	1.85	1.86	1.84	1.81	1.76	1.69
Solids-not-fat (lb.)	3.01	3.54	3.99	4.33	4.54	4.61	4.63	4.60	4.55	4.4
Total solids (lb.)	4.17	4.96	5.62	6.11	6.39	6.47	6.47	6.41	6.31	6.14
Fat in total solids (%)	27.8	28.6	29.0	29.1	29.0	28.8	28.5	28.3	27.9	27.5

Number of analyses = 370.

Average number of days since calving = 52 days (2-9 years).

Table IV. *British Friesian Cows.*

Age (years)	2	3	4	5	6	7	8	9	10
Average No. of days since calving	49	65	50	60	62	53	53	68	78
No. of analyses	46	42	76	58	58	34	18	12	8
Yield (lb.)	39.0	47.0	49.5	55.6	58.5	59.7	62.1	63.2	63.5
Fat (%)	3.55	3.62	3.67	3.68	3.64	3.57	3.49	3.40	3.31
Solids-not-fat (%)	8.89	8.89	8.87	8.85	8.82	8.80	8.78	8.75	8.73
Fat (lb.)	1.38	1.70	1.82	2.05	2.13	2.13	2.17	2.15	2.10
Solids-not-fat (lb.)	3.47	4.17	4.39	4.92	5.16	5.26	5.45	5.53	5.54
Total solids (lb.)	4.85	5.87	6.21	6.97	7.29	7.39	7.62	7.68	7.64
Fat in total solids (%)	28.5	29.0	29.3	29.4	29.2	28.8	28.5	28.0	27.5

Number of analyses = 352.

Average number of days since calving = 56 (2-8 years).

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Table V. *Ayrshire Cows.*

Age (years)	2	3	4	5	6	7	8	9
Average No. of days since calving	28	49	25	41	53	29	63	81
No. of analyses	74	30	26	50	42	28	20	10
Yield (lb.)	36.0	40.5	44.5	47.5	49.2	49.9	49.5	47.0
Fat (%)	4.31	4.33	4.28	4.18	4.04	3.90	3.72	3.52
Solids-not-fat (%)	9.26	9.20	9.13	9.07	9.01	8.94	8.88	8.82
Fat (lb.)	1.55	1.75	1.90	1.98	1.98	1.95	1.84	1.65
Solids-not-fat (lb.)	3.33	3.72	4.06	4.30	4.43	4.46	4.39	4.15
Total solids (lb.)	4.89	5.47	5.96	6.28	6.41	6.41	6.23	5.80
Fat in total solids (%)	31.8	32.0	31.9	31.5	30.9	30.4	29.6	28.5

Number of analyses = 280.

Average number of days since calving = 41 (2-8 years).

Table VI. *Jersey Cows.*

Age (years)	2	3	4	5	6	7	8	9	10	11	12
Average No. of days since calving	77	96	95	114	138	114	110	121	111	121	129
No. of analyses	130	176	244	212	150	146	82	80	30	14	10
Yield (lb.)	26.0	28.9	31.5	32.7	33.4	33.7	33.7	33.6	33.5	33.5	33.4
Fat (%)	5.20	5.22	5.24	5.23	5.20	5.16	5.08	5.01	4.95	4.88	4.83
Solids-not-fat (%)	9.55	9.37	9.30	9.26	9.23	9.21	9.20	9.19	9.19	9.18	9.17
Fat (lb.)	1.35	1.51	1.65	1.71	1.74	1.74	1.71	1.68	1.66	1.63	1.61
Solids-not-fat (lb.)	2.48	2.71	2.93	3.02	3.08	3.10	3.10	3.09	3.08	3.07	3.06
Total solids (lb.)	3.83	4.22	4.58	4.73	4.82	4.84	4.81	4.77	4.74	4.70	4.67
Fat in total solids (%)	35.3	35.8	36.0	36.2	36.1	36.0	35.5	35.2	35.0	34.7	34.5

Number of analyses = 1274.

Average number of days since calving = 108 (2-10 years).

Table VII. *Guernsey Cows.*

Age (years)	2	3	4	5	6	7	8	9	10	11	12
Average No. of days since calving	84	81	78	81	92	85	89	106	99	71	91
No. of analyses	84	70	92	82	78	52	48	32	12	14	8
Yield (lb.)	26.4	30.2	31.8	32.7	33.0	33.0	33.0	32.9	32.9	32.8	32.7
Fat (%)	4.90	4.92	4.90	4.86	4.81	4.75	4.67	4.58	4.50	4.45	4.41
Solids-not-fat (%)	9.46	9.37	9.30	9.24	9.20	9.17	9.15	9.14	9.13	9.12	9.12
Fat (lb.)	1.29	1.49	1.56	1.59	1.59	1.57	1.54	1.51	1.48	1.46	1.44
Solids-not-fat (lb.)	2.50	2.83	2.96	3.02	3.03	3.02	3.02	3.00	3.00	2.99	2.98
Total solids (lb.)	3.80	4.32	4.52	4.61	4.62	4.59	4.56	4.51	4.48	4.45	4.42
Fat in total solids (%)	34.2	34.5	34.5	34.5	34.4	34.2	33.8	33.5	33.0	32.8	32.6

Number of analyses = 572.

Average number of days since calving = 89 (2-9 years).

Table VIII. *Kerry Cows.*

Age (years)	2	3	4	5	6	7	8	9	10	11	12
Average No. of days since calving	61	84	68	56	74	83	85	93	66	69	94
No. of analyses	50	32	34	46	36	42	28	26	12	14	10
Yield (lb.)	21.0	26.0	30.5	33.6	35.8	36.2	36.1	35.8	35.2	34.5	34.0
Fat (%)	4.48	4.52	4.49	4.38	4.22	4.10	4.00	3.91	3.85	3.77	3.72
Solids-not-fat (%)	9.30	9.30	9.23	9.16	9.10	9.06	9.02	8.99	8.97	8.96	8.95
Fat (lb.)	0.94	1.17	1.37	1.48	1.51	1.48	1.44	1.40	1.35	1.30	1.26
Solids-not-fat (lb.)	1.95	2.42	2.81	3.08	3.26	3.28	3.25	3.22	3.16	3.10	3.04
Total solids (lb.)	2.89	3.59	4.18	4.56	4.77	4.76	4.69	4.62	4.51	4.40	4.30
Fat in total solids (%)	32.5	32.6	32.8	32.5	31.6	31.1	30.7	30.3	30.0	29.6	29.3

Number of analyses = 330.

Average number of days since calving = 76 (2-9 years).

Table IX. *Dexter Cows.*

Age (years)	2	3	4	5	6	7	8	9
Average No. of days since calving	52	116	113	117	129	130	93	133
No. of analyses	14	22	20	20	32	22	18	6
Yield (lb.)	18.0	21.2	23.7	25.9	27.5	28.5	28.5	27.6
Fat (%)	4.42	4.47	4.44	4.34	4.16	3.99	3.83	3.67
Solids-not-fat (%)	9.30	9.30	9.24	9.14	9.05	8.96	8.89	8.80
Fat (lb.)	0.79	0.95	1.05	1.12	1.14	1.14	1.09	1.01
Solids-not-fat (lb.)	1.67	1.97	2.19	2.37	2.49	2.55	2.54	2.43
Total solids (lb.)	2.46	2.92	3.24	3.49	3.63	3.69	3.63	3.44
Fat in total solids (%)	32.1	32.5	32.4	32.1	31.4	31.0	29.5	29.4

Number of analyses = 154.

Average number of days since calving = 107 (2-8 years).

## YIELD.

The yield of milk of all breeds of cows at first increases rapidly with age, reaches a maximum and then gradually falls off. The age at which this maximum is reached differs slightly for different breeds, as also does the length of time during which the greatest yield is given.

This is illustrated in Table X, which shows for each breed the percentage of the maximum yield given by cows of various ages.

Unfortunately there is insufficient data available in the majority of cases to make definite conclusions possible as to the rate at which the yield falls off with age after the maximum has been reached.

The relative effect of age on the yields of milk of Ayrshire cows agrees with that given by Tocher (3), although, as would be anticipated, the actual values of the yields differ.

## 428 *Influence of Cow's Age on the Quality of Milk*

Table X.

Breed	2	3	4	5	6	7	8	9	10	11	12
Dairy Shorthorn	59.4	72.7	83.4	91.0	96.2	98.8	100	100	99.6	99.0	97.7
Red Poll	70.3	83.0	90.5	94.8	97.4	99.2	99.8	100	99.8	99.1	—
Lincoln Red	62.7	74.1	84.1	91.9	96.8	99.1	100	100	99.5	97.8	—
British Friesian	61.4	74.0	78.0	87.6	92.2	94.0	97.8	99.6	100	—	—
Ayrshire	72.2	81.2	89.2	95.2	98.7	100	99.3	94.3	—	—	—
Jersey	77.1	85.8	93.5	97.1	99.2	100	100	99.7	99.5	99.5	99.2
Guernsey	80.0	91.6	96.5	99.2	100	100	100	99.7	99.7	99.5	99.2
Kerry	58.0	65.0	84.3	92.8	99.0	100	99.7	99.0	97.3	95.6	94.0
Dexter	63.2	74.5	83.2	91.0	96.6	100	100	97.0	—	—	—

The Shorthorn breeds attain their maximum yield rather later than the Jersey and Guernsey cows, and their yields show a greater variation with age. Thus two year old Shorthorn cows give only 60 per cent. of the yield given by the mature cow, whereas two year old Jersey and Guernsey cows give 80 per cent.

In the former paper (1), (p. 128) attention was drawn to the fact that the records available for the Jersey cows showed that the yield of milk was maintained until the later stages of the lactation period studied and it was suggested that this might be due to an uneven age distribution. A reference to Table VI justifies this supposition, as the average number of days since calving for each age tends to increase with the age. Hence there are two opposing factors in operation, the advancement of the lactation period reducing the yield of milk, and the increased age of the cows tending to increase the yield. The result is that records for the Jersey cows indicate that they apparently maintain their yield of milk with advance in lactation to a greater extent than any other herd.

### FAT.

The percentage of fat in the milk of all breeds of cows at first increases slightly with age and then decreases, and for all breeds the quality of milk of very young cows is richer than that of cows over six or seven years of age. The age at which the milk contains the maximum percentage of fat differs very slightly for various breeds, thus Shorthorn cows give the highest percentage of fat in the milk when they are four and five years of age, whereas Guernsey, Kerry and Dexter cows attain their maximum at three years.

The actual weight of fat given by cows of each breed rises to a maximum between the 5th and 8th year and then decreases. The percentage of fat in the total solids of the milk shows but small variation, but in general it gradually rises to a maximum between the 5th and 8th year and then diminishes.

The effect of age on the fat percentage of the milk is shown in Table XI. To facilitate comparisons, the maximum fat content for each breed is given as 100.

Table XI.

Breed	2	3	4	5	6	7	8	9	10	11	12
Dairy Shorthorn	95.7	98.5	100	100	98.3	96.7	95.0	93.1	91.3	89.0	86.9
Red Poll	96.8	99.7	100	97.5	95.0	93.1	92.3	91.8	91.6	91.1	—
Lincoln Red	96.8	98.7	100	99.8	98.4	96.8	95.0	93.4	91.5	89.3	—
British Friesian	96.6	98.4	99.8	100	98.9	97.1	94.9	92.4	90.0	—	—
Ayrshire	99.6	100	98.9	96.6	93.4	90.1	86.0	81.4	—	—	—
Jersey	99.3	99.7	100	99.9	99.3	98.4	97.0	95.6	94.5	93.2	92.2
Guernsey	99.7	100	99.6	99.3	97.8	96.6	95.0	93.1	91.5	90.5	89.7
Kerry	99.1	100	99.4	96.9	93.4	90.7	88.5	86.5	85.2	83.4	82.4
Dexter	98.9	100	99.3	97.2	93.1	89.3	85.7	82.1	—	—	—

## SOLIDS-NOT-FAT.

The percentage of solids-not-fat decreased continuously with age, although in some cases the decrease is almost imperceptible until after the 3rd year, whilst the actual weight of solids-not-fat increases until about the 8th year.

## CONCLUSION.

The influence of both the age of the cow and the period of lactation upon the yield and quality of the milk have been separately investigated, and relationship curves have been obtained. It is proposed in a subsequent communication to deduce for each breed the joint relationship between the variable factors, age, period of lactation, yield and quality of milk. This will furnish a means of comparing the milking qualities of cows of various breeds, at various ages, and at various stages of their lactation periods. Up to the present the adjustment of points made in Milking Trials, such as those conducted at the London Dairy Show by the British Dairy Farmers' Association for breed, variations in period of lactation, etc., have been purely empirical and have been open to considerable criticism.

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## BIOCHEMISTRY OF WATER-LOGGED SOILS.

## PART I.

THE EFFECT OF WATER-LOGGING ON THE DIFFERENT FORMS  
OF NITROGEN, ON THE REACTION, ON THE GASEOUS  
RELATIONSHIPS, AND ON THE BACTERIAL FLORA<sup>1</sup>.

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(With Eight Graphs.)

WATER-LOGGING of soils is a common phenomenon, especially in the tropics where the rains are heavy and rivers frequently overflow their banks or lakes burst their bounds; vast areas often remain for long periods submerged. The rice fields—which cover an area of about eighty million acres in India alone—have to be maintained for months in the swamp state. The chemical and biological changes which attend such conditions are therefore of great economic importance and considerable scientific interest.

The scientific literature on the subject is comparatively limited. In 1881 Warington<sup>(26)</sup> carried out some laboratory trials and found that he could not completely recover added nitrates when the soil remained covered with water. Nagaoka<sup>(17)</sup> observed that nitrites were formed as a result of heavy dressings of nitrates to rice fields. Daikuhara and Imasaki<sup>(4)</sup> noted considerable reduction in the soluble nitrogen. Kelly<sup>(12)</sup> observed that swamping led to considerable fall in nitrate content and that the nitrites formed were toxic to plants. Oelsner<sup>(19)</sup> stated that increased moisture content of soils beyond 20 per cent. led to inevitable loss of nitrates and that at 40–50 per cent. even larger quantities of total nitrogen were lost to the soil.

Harrison and Iyer<sup>(11)</sup> carried out researches on the gases of swamp rice soils and found that methane, hydrogen, carbon dioxide and nitrogen were the chief gases present in the soil, but that by the combined action of certain aerobic bacteria, green algae and the roots of plants, they

<sup>1</sup> Parts I to IV of this series were submitted to the Royal Agricultural Society of England in 1926 as a Thesis, for which the author was awarded the Research Medal of that Society.

were oxidised and decarbonised, resulting in the evolution of only free oxygen and nitrogen.

It is desirable to have detailed knowledge of the more fundamental changes and the object of the work described in this series of papers is to obtain quantitative data regarding the effect of water-logging on the various forms of nitrogen, the oxygen, carbon dioxide and acidity relations, the bacterial numbers and other attendant biological phenomena.

### *Experimental.*

The soils taken for detailed investigation were (1) Soil from Plot 1,0 of Barnfield, Rothamsted, which has been under root-cultivation since 1856 and receives 14 tons of dung to the acre annually; (2) Garden soil from Central Farm, Coimbatore, South India.

The samples were air-dried, freed from undecomposed plant residues, passed through a 1 mm. sieve, shaken well with water (100 parts of soil in 250 parts of water) and incubated for 40 days, the Rothamsted soil at 20° C. and the Indian soil at 35° C. Samples were taken at three-day intervals for analysis.

### *The Effect of Water-logging on the Nitrogen Compounds.*

*Gaseous ammonia* evolved from the soils was estimated by absorption in 0.004*N* acid and back-titration against alkali of the same strength.

Very minute but definite quantities (1.6 P.P.M. from the Rothamsted soil and 1.2 P.P.M. from the Indian soil) were evolved within the first 24 hours, but after that time the amounts evolved were not appreciable (Graph 1).

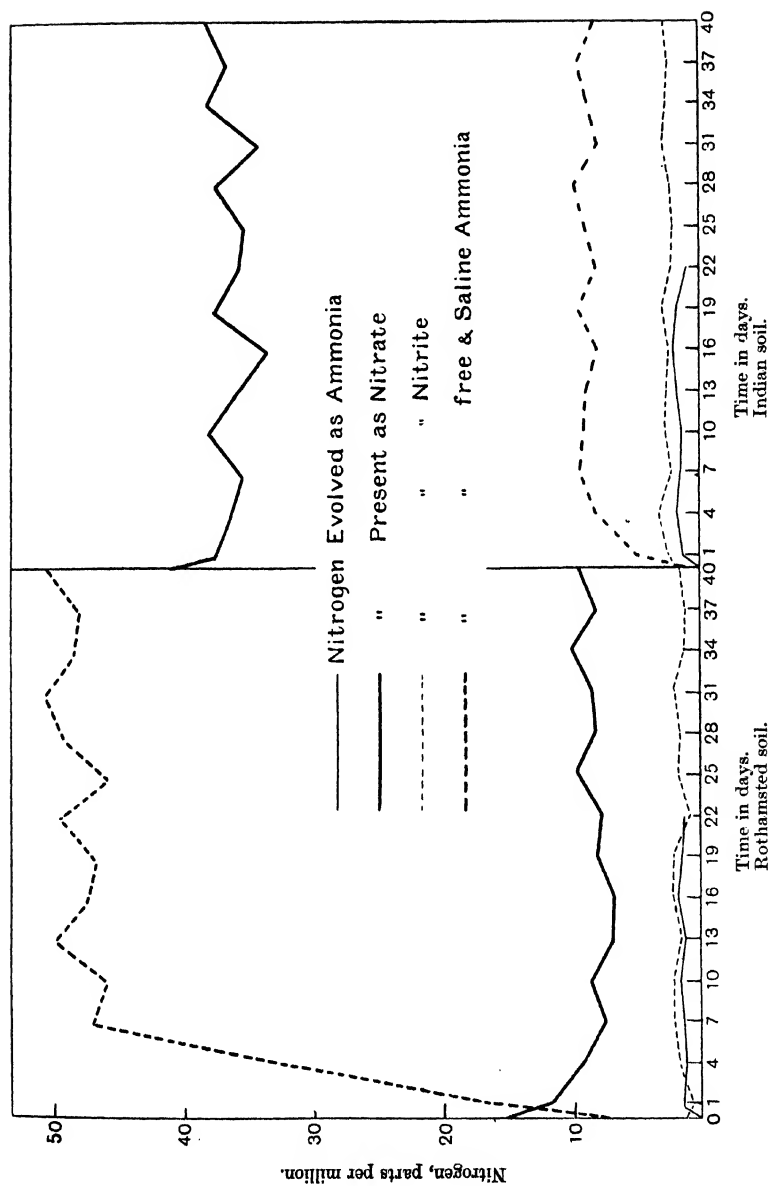
*Free and saline ammonia* were estimated by an aeration method (20).

A very distinct increase was noticeable even at the end of the first 24 hours. The rise continued up to the end of the seventh day, after which the variations were all within the limits of experimental error (Graph 1).

The rate of formation of ammonia in the Rothamsted soil was about five times that in the Indian soil, being 39 P.P.M. in seven days in the former against only 8 P.P.M. in the latter. In spite of the difference in temperature, ammonia-formation proceeded vigorously in both the soils during the same period of time and then came practically to a stop.

The mechanism of the production of ammonia cannot be purely chemical: amides exist in the soil only in small quantities and are not decomposed even by a 4 per cent. solution of sodium carbonate (20).

*Nitrites* were estimated by Davisson's titration method (5).



Graph 1. Changes in the different forms of Nitrogen in water-logged soils.

The two soils showed only very slight increases in the early stages (1.1 P.P.M. at the end of seven days for the Rothamsted soil and 1.4 P.P.M. at the end of four days for the Indian soil), but later a succession of rises and falls was noted, most of which lie within the range of experimental error (Graph 1). A critical examination of the figures shows that although the general average appears to be increased as the result of the water-logging there is not sufficient evidence to conclude that the rise in nitrite-content is real.

*Nitrates* together with nitrites were estimated by the Devarda's Alloy method<sup>(24)</sup>. The nitrites having been determined separately, the nitrates were obtained by difference. Both the soils showed marked reduction in nitrates (7.1 P.P.M. in the Rothamsted soil and 5.7 P.P.M. in the Indian soil) during the first seven days (Graph 1). The quantities present later seemed to vary only slightly.

It is possible that a part of the nitrate lost was converted into nitrite. A portion may have been reduced to ammonia; but the latter is produced in such large quantities that it could not have come from the nitrates alone. Some nitrates may have been assimilated by micro-organisms or reduced to nitrogen gas.

*Total nitrogen* was determined by the Kjeldahl method.

As has already been pointed out<sup>(21)</sup> the relatively high standard error detracts much from the value of the determinations: but these estimations were carried out in view of the fact that, by adopting a similar method, cases of marked loss of nitrogen have been recorded<sup>(19)</sup>.

A study of the figures showed that they did not vary significantly. The average content of total nitrogen was 2439 P.P.M. for Rothamsted soil and 888.5 P.P.M. for Indian soil. The values for different times of sampling varied irregularly about these means, and in no case did the deviation exceed twice the standard error of difference<sup>1</sup> which was  $\pm 27.2$  P.P.M. for Rothamsted soil and  $\pm 23.0$  P.P.M. for Indian soil. There is thus no evidence to suggest that as a result of water-logging the total nitrogen of the soils suffered any change in amount.

#### *Comparative Study of Nitrogen Changes in various Indian Soils.*

It has so far been noted that as a result of the water-logging there were (a) a distinct increase in free and saline ammonia, (b) significant though slight reduction in nitric nitrogen and (c) no diminution at all in total nitrogen. In order to see if these phenomena were general and could be observed on soils which become or are apt to become water-

<sup>1</sup>  $\sqrt{2} \times \text{Standard Error.}$

logged frequently, representative specimens from the various provinces of the Indian Empire<sup>1</sup> were obtained and studied for the changes in the three forms of nitrogen.

Free and saline ammonia were estimated in this series by the McLean and Robinson method (15).

A statistical study (7) of the results (see Table I) shows that in spite of the great diversity in origin and character of the soils the increase in free and saline ammonia content was quite pronounced in all cases, even at the end of the third day. The production continued at more than double the rate up to the end of the tenth day.

The formation of ammonia went on much more vigorously in certain soils than in others. There is no correlation whatever between the rate of production and the total nitrogen content or the amount of ammonia originally present.

Table I. *Table showing increased Production of Free and Saline Ammonia in different Indian Soils as a result of Water-logging.*

No.	Origin of sample	Total Nitrogen	Nitrogen as parts per million Free and Saline Ammonia			
			At start	3rd day	7th day	10th day
I.	Dera Ghazi Khan, Punjab	679.7	13.4	21.5	30.6	39.8
II.	Jadiala Baghwala, Punjab	345.3	19.6	15.0	21.3	29.7
III.	Chicoki Malian, Punjab	336.9	10.0	21.7	35.8	46.1
IV.	Chuhur Khana, Punjab	863.3	13.8	24.2	37.3	46.8
V.	Karimganj, Assam	772.4	15.5	16.3	20.3	27.5
VI.	Pausra, Punjab	643.5	11.2	18.9	26.4	36.9
VII.	Marh Bhangwan, Punjab	282.9	10.7	19.4	27.9	35.3
VIII.	Chicharianwali, Punjab	334.4	21.5	22.2	26.9	32.1
IX.	Titabar, Assam	2748.7	7.4	13.4	20.2	25.8
X.	Kaliganj, Bengal	1100.9	13.9	22.0	28.9	36.2
XI.	Dacca, Bengal	1233.1	21.8	26.4	32.0	39.9
XII.	Yessgaon No. 1, Bombay	425.6	9.3	8.8	12.9	16.6
XIII.	Yessgaon No. 2, Bombay	404.2	9.5	9.9	13.4	16.9
XIV.	Kopergaon, Bombay	575.8	9.4	16.5	21.8	26.8
XV.	Malad No. 1, Bombay	711.5	4.9	16.7	23.3	30.2
XVI.	Malad No 2, Bombay	744.9	12.4	12.7	16.9	19.3
XVII.	Karjat, Bombay	570.6	6.7	12.7	20.1	25.2
XVIII.	Hmawbi, Burma	766.8	12.4	12.4	15.4	17.8
XIX.	Mandalay, Burma	545.1	19.0	23.0	30.5	36.3
XX.	Anakapalli, Madras	866.2	59.2	62.3	67.8	71.3
XXI.	Coimbatore F Block	1460.2	27.7	52.3	65.6	76.9
XXII.	Coimbatore M Block	630.8	23.0	29.3	36.1	44.8
XXIII.	Tirur, Madras	2556.6	34.1	40.5	45.7	51.3
XXIV.	Sholavandan, Madras	522.0	27.2	28.7	32.1	34.8

Average increase ( $\bar{x}$ ) ÷ Standard Error =  $t$ .  $t = 2$  is taken as significant.

Values of  $t$ : 0-3rd day = 4.7; 3-7th day = 11.1; 7-10th day = 11.0.

<sup>1</sup> The author takes this opportunity to thank the Directors of Agriculture of the various provinces for their willing response to his request for samples.

## 434 *Biochemistry of Water-logged Soils. Part I*

*Nitrates.* A study of the data (Table II) shows that whilst the reduction in the nitrate content on the third day was not marked, that at the end of the seventh day was significant. There was practically no difference between the quantities present on the seventh and the tenth days.

The amounts lost on water-logging do not seem to bear any relation to either the respective total-nitrogen contents or to the quantities of nitrates originally present.

Table II. *Variations in the Nitrate-Content of Different Indian Soils when Water-logged.*

No.	Origin of sample	Total Nitrogen	Nitrogen as parts per million Nitric Nitrogen			
			At start	3rd day	7th day	10th day
I.	Dera Ghazi Khan	679.7	20.8	19.8	20.2	19.4
II.	Jadiala Baghwala	345.3	5.7	1.5	2.1	1.9
III.	Chicoki Malian	336.9	44.9	35.6	36.8	36.2
IV.	Chuhur Khana	863.3	239.5	247.5	240.2	232.3
V.	Karimganj	772.4	7.9	8.2	7.6	8.0
VI.	Pausra	643.5	13.7	12.6	13.0	12.8
VII.	Marh Bhangwan	282.9	34.3	34.6	33.8	34.2
VIII.	Chicharianwali	334.4	9.9	9.3	8.8	9.2
IX.	Titabar	2748.7	1.7	4.5	3.3	2.9
X.	Kaliganj	1100.9	9.9	12.1	10.9	11.2
XI.	Dacca	1233.1	5.1	2.9	3.6	3.2
XII.	Yessgaon No. 1	425.6	1.4	1.1	1.0	1.2
XIII.	Yessgaon No. 2	404.2	1.6	1.2	1.4	1.0
XIV.	Kopergaon	575.8	14.4	12.8	10.2	9.6
XV.	Malad No. 1	711.5	8.2	7.1	7.3	6.8
XVI.	Malad No. 2	744.9	8.4	8.0	7.6	7.5
XVII.	Karjat	570.6	3.0	2.0	1.3	1.1
XVIII.	Hmawbi	766.8	2.3	3.0	2.7	2.1
XIX.	Mandalay	545.1	5.3	2.3	2.0	2.7
XX.	Anakapalli	866.2	6.0	4.6	4.8	4.4
XXI.	Coimbatore F Block	1460.2	4.2	3.1	3.3	3.8
XXII.	Coimbatore M Block	630.8	12.2	11.1	10.5	10.3
XXIII.	Tirur	2556.6	14.2	8.6	7.9	8.2
XXIV.	Sholavandan	522.0	7.3	6.2	5.7	5.4

Average diminution  $(\bar{x}) \div \text{Standard Error} = t$ .  $t=2$  is taken as significant.

Values of  $t$ : 0-3rd day = 1.4; 3-7th day = 1.8; 7-10th day = 1.3;

0-7th day = 3.3; 0-10th day = 4.7.

*Total nitrogen.* An analysis of the results (Table III) shows that in spite of the marked differences between the individual data the average variances are insignificant. There is no evidence to show that any loss in total nitrogen followed as a result of mere water-logging.

Table III. *Table showing the total Nitrogen Contents at different times in Water-logged Soils.*

No.	Origin of the soil	Total Nitrogen as parts per million		
		At start	15th day	30th day
I.	Dera Ghazi Khan	679.7	663.6	670.3
II.	Jadiala Baghwala	345.3	359.8	340.1
III.	Chicoki Malian	336.9	325.7	331.2
IV.	Chuhur Khana	863.3	871.8	866.7
V.	Karimganj	772.4	756.2	761.5
VI.	Pausra	643.5	662.7	631.2
VII.	Marh Bhangwan	282.9	275.7	277.1
VIII.	Chicharianwali	334.4	326.6	329.3
IX.	Titabar	2748.7	2762.8	2756.2
X.	Kaliganj	1100.9	1112.3	1116.5
XI.	Dacca	1233.1	1221.2	1238.6
XII.	Yessgaon No. 1	425.6	431.3	423.2
XIII.	Yessgaon No. 2	404.2	411.8	421.2
XIV.	Kopergaon	575.8	569.1	588.2
XV.	Malad No. 1	711.5	706.8	728.7
XVI.	Malad No. 2	744.9	751.1	741.6
XVII.	Karjat	570.6	562.7	566.0
XVIII.	Hmawbi	766.8	742.3	755.1
XIX.	Mandalay	545.1	561.1	532.7
XX.	Anakapalli	866.2	851.6	881.8
XXI.	Coimbatore F Block	1460.2	1479.1	1488.7
XXII.	Coimbatore M Block	630.8	610.1	600.3
XXIII.	Tirur	2556.6	2536.8	2563.5
XXIV.	Sholavandan	522.0	539.5	509.9

Average diminution ( $\bar{x}$ )  $\div$  Standard Error  $= t$ .  $t = 2$  is taken as significant.

Values of  $t$ : 0-15th day - 0.4; 15-30th day - 0.3.

### *The Effect of Water-logging on the Reaction.*

The hydrogen-ion concentration was determined by the method of drop-ratios(8), using the filtrate obtained from the water-logged soil after shaking up. The results are plotted in Graph 2.

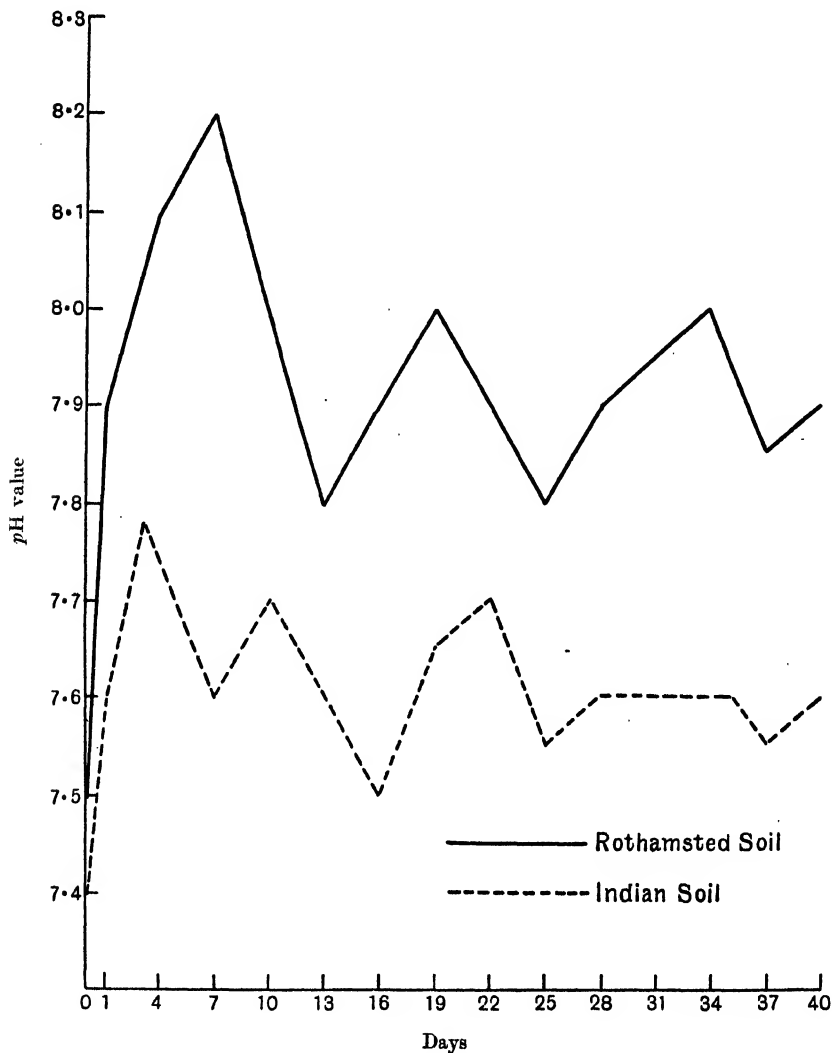
There was a distinct rise in the pH values of the liquid, notably during the early stages (7.5-8.2 in seven days in the Rothamsted soil and 7.4-7.8 in four days in the Indian soil). There seemed to be a slight decrease after that time, but the figures still remained higher than at the beginning. The effects were much more marked in the Rothamsted than in the Indian soil.

Gillespie(9) has already remarked that water-logging tends to make soils less intensely acid. It should be observed that the rise in the pH value was more or less simultaneous with the increase in the free and saline ammonia content of the soils. An examination of the data showed clearly that there was a distinct positive correlation between the pH values and the logarithms of the corresponding amounts of free and saline ammonia.



*The Effect of Water-logging on the Gaseous Relations.*

*Oxygen absorption.* Gillespie<sup>(9)</sup> has observed that there is a reduction potential set up in water-logged soils. In order to ascertain if that could

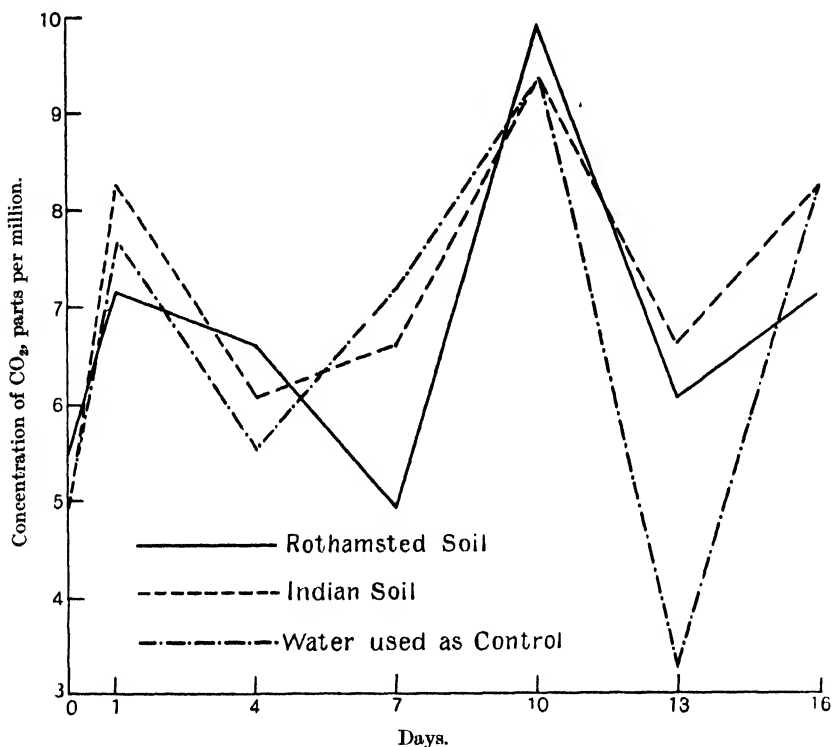


Graph 2. Changes in the Hydrogen-ion concentration.

be due to the formation of soluble reducing matter, a series of determinations of the oxygen absorbed by the soil extract at various times was carried out<sup>(16)</sup>.

The results showed that whilst at the end of the first 24 hours there was a significant rise in oxygen absorption by the extract of one soil there was a similar fall in the absorption by the other. The figures for the later days showed marked rises and falls, but did not indicate any real difference in the absorption.

There is no evidence to suggest any appreciable variation in the absorption of oxygen by the extracts from the two water-logged soils.



Graph 3. Variations in the quantities of dissolved  $\text{CO}_2$ .

*Production of carbon dioxide.* Previous work(18) has shown that the production of ammonia from added protein matter in soils is generally accompanied by a corresponding evolution of carbon dioxide. In order to see if any similar correlation could be observed in the water-logged soils a series of determinations of the carbon dioxide formed at various times were carried out. The estimations were made by absorbing the gas in baryta contained in a flask connected to that containing the soil,

and titrating the excess of the baryta against standard acid. The amount of carbon dioxide given off was found to be quite small.

A study of the quantities present in the dissolved state showed that though varying slightly they did not differ significantly from the quantities present in a sample of distilled water maintained under identical conditions as control. (Graph 3.)

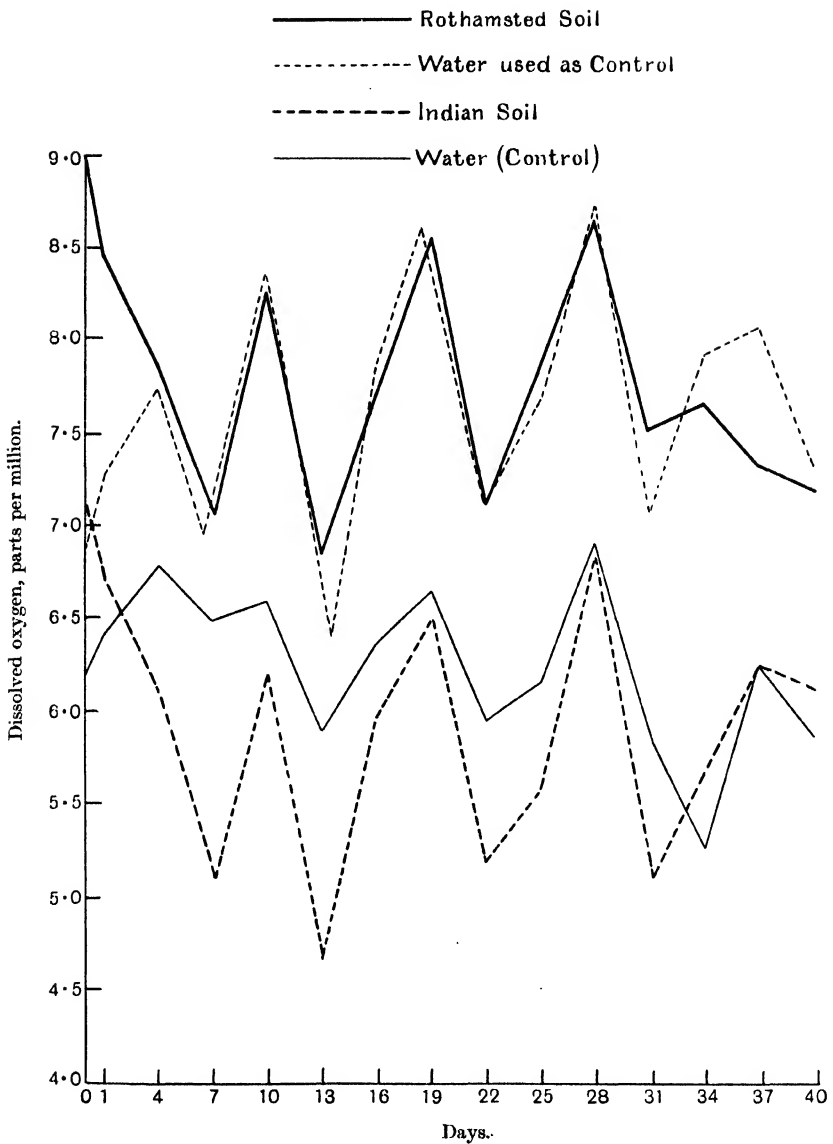
It has already been pointed out that the increased production of ammonia could not be a purely chemical process and it was suggested that it might have been due to biological activity. But if the reaction had been brought about by any of the active ammonifying organisms (14 and 2) it must have been accompanied by the evolution of carbon dioxide. Since in the present case there is practically no carbon dioxide produced, the possible action of such organisms is precluded.

#### *Dissolved Oxygen of the Surface Water.*

Harrison and Iyer<sup>(11)</sup> have suggested that a probable source of oxygen to the swamp soils is that present dissolved in the surface water. In order to obtain an idea of the oxygen relations of the supernatant water a series of determinations of dissolved oxygen in it at different times was made. The estimations were made by Rideal and Stewart's modification of Winkler's method<sup>(22)</sup> with correction for the reducing matter of the soil.

A study of the results (Graph 4) shows that marked fluctuations in the content of dissolved oxygen occurred during the period of the experiments, but that these fluctuations were always in the same direction for the control and the water above the soil samples. The fluctuations were therefore due to variations in external conditions with time, and not to any soil factor.

In the case of the Rothamsted soil, which was kept at laboratory temperature, the individual values for the soil water and the control do not differ significantly, the differences being small and indiscriminately positive and negative. With the Indian soil, however, which was kept at a temperature of 35° C., the dissolved oxygen content of the soil water was less than that of the control at nearly every time of sampling. This appears to indicate that the latter soil was absorbing oxygen at a rate slightly faster than that at which it could be replenished from the atmosphere. If such absorption occurred with the Rothamsted soil it was so slow, at the lower temperature used, that its effect was masked by the supply of dissolved oxygen from the air.



Graph 4. Variations in the Dissolved Oxygen of the surface water.

*The Diffusion of Oxygen from the Surface Water into the Soil.*

In order to study quantitatively the diffusion of oxygen from the surface water into the soil 250 gm. lots of the soils were taken in Winchesters, saturated with water and partially deoxygenated by standing for a week connected with jars containing alkaline pyrogallol. The Winchesters were then filled with water which had been standing for some time under laboratory conditions and the oxygen content of which was steady, and tightly stoppered. After standing for 24 hours at laboratory temperature for each soil the oxygen content at successive one-inch depths was determined. The results were as follows:

Table IV.

Oxygen present originally in the water 84.7 P.P.M.		Bar. P. 777.3 mm. Temp. 1.57° C.		
Depth from the surface of the water (in inches)	Oxygen as parts per million			
	Rothamsted soil		Indian soil	
	Oxygen present	Oxygen lost	Oxygen present	Oxygen lost
1	8.35	0.12	8.41	0.06
2	8.04	0.43	8.28	0.19
3	7.63	0.84	7.78	0.69
4	6.58	1.89	6.91	1.56
5	5.57	2.90	6.46	2.01
6	5.05	3.42	5.35	3.12
7	4.75	3.72	5.08	3.39
8	4.52	3.95	4.81	3.66
9	4.47	4.0	4.63	3.84

The results show that the oxygen present in the soil was used up; dissolved oxygen from the surface water diffused downward and took its place.

An equation expressing the oxygen concentration-gradient in the water above a soil is

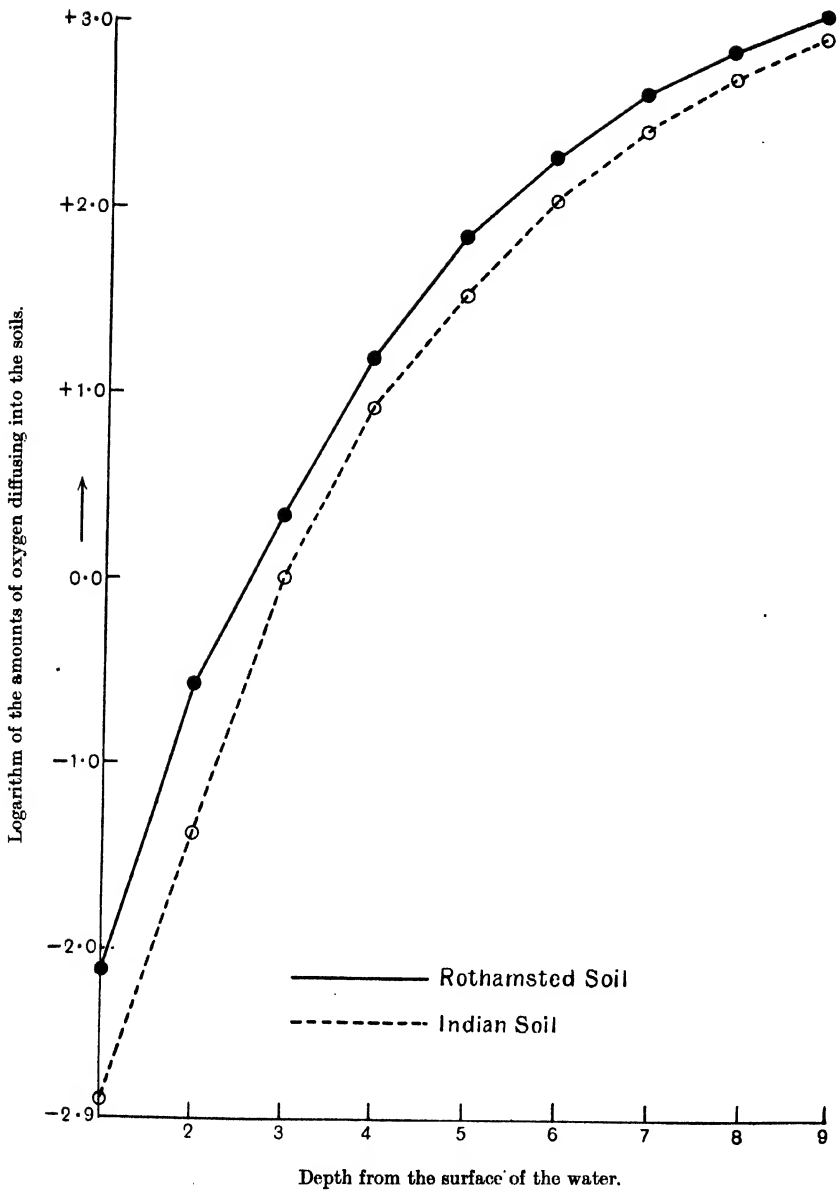
$$\log_e Y = K + pd + qd^2 + rd^3,$$

where  $Y$  is the decrement of oxygen content at a depth  $d$  below the surface,  $K$  is a constant for the soil, and  $p$ ,  $q$  and  $r$  are constants independent of the soil, and determined by the rate of diffusion of oxygen through the liquid. In Graph 5 the logarithms of the decrements of oxygen content are plotted against the corresponding depths (Table IV). The curves obtained are fairly uniform and correspond closely to the equations:

$$\log_e Y = 1.264 + 0.6x - 0.09x^2 + 0.0065x^3 \text{ (for Rothamsted soil),}$$

$$\log_e Y = 0.912 + 0.6x - 0.09x^2 + 0.0065x^3 \text{ (for Indian soil).}$$

The actual quantities of oxygen present at any time at any given depth



Graph 5. The diffusion of oxygen from the surface water into the soil.

are determined by the rate of absorption of oxygen by the soils. The values of  $K$  for the two soils (1.264 for the Rothamsted soil and 0.912 for the Indian soil) show that the rate of absorption is greater for the former soil, when the temperature is the same for both.

The absorption of oxygen by these soils would appear to be non-biological in character, since it has been shown (p. 438) that there is no corresponding production of carbon dioxide.

#### *The Effect of Water-logging on the Bacterial Flora.*

Bacterial counts were made by plating on Thornton's Standardised Agar<sup>(25)</sup>. The results are plotted in Graph 6.

There was a perceptible fall (37 to 26 M.P.G. in the Rothamsted soil and 25 to 16 M.P.G. in the other) in the numbers present at the end of the first twenty-four hours. Later the counts show a succession of rises and falls, many of which are significant. This observation is very similar to the one made during short period counts on arable soils<sup>(2)</sup>. It is possible that in both cases the variations are caused by the presence of protozoa which are known to be particularly numerous in wet soils<sup>(3)</sup>.

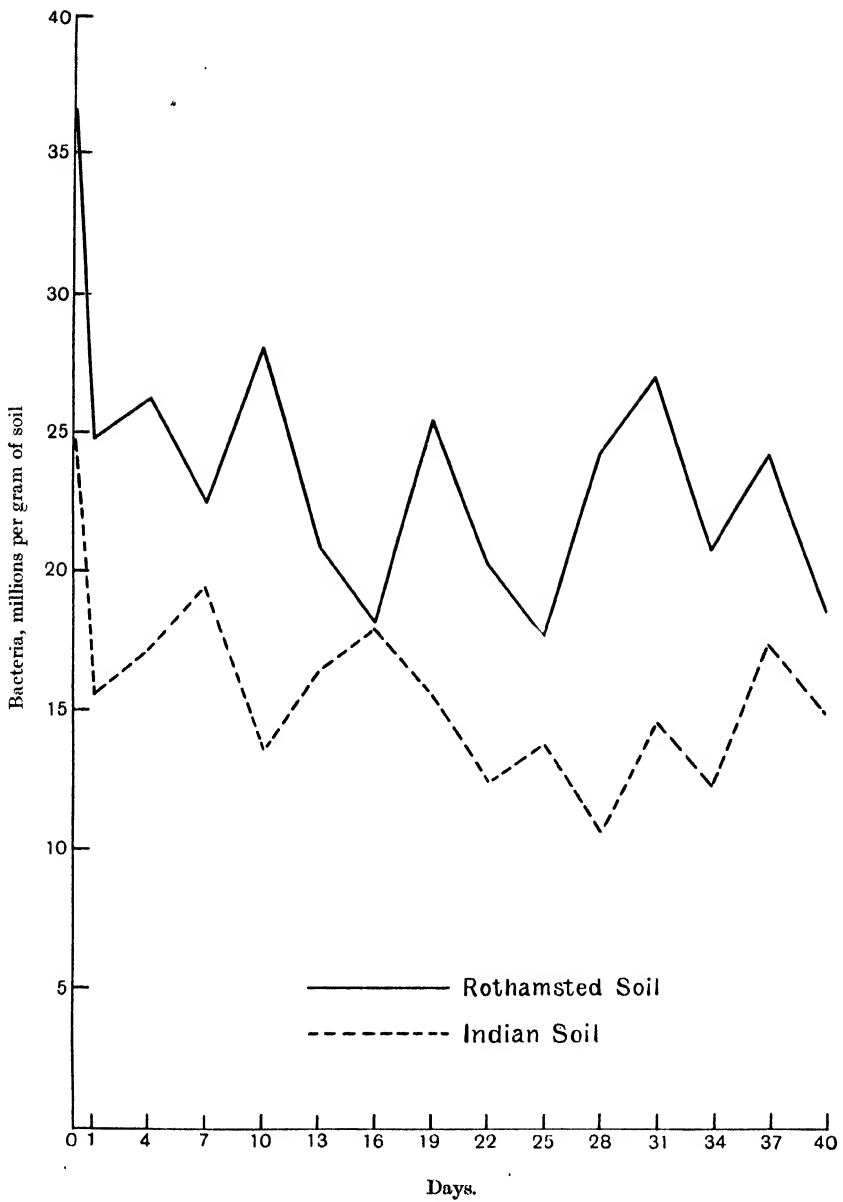
*Giltay's Agar*. In view of the fact that water-logging has frequently been associated with denitrification, platings were made under aerobic and anaerobic conditions on Giltay's Agar, which is a favourable medium for the isolation and development of denitrifying organisms<sup>(10)</sup>.

The numbers of bacteria observed in the aerobic platings on this medium were much less than on Thornton's Agar and were highly inconsistent, the standard error being  $\pm 10.9$  per cent. (Graph 7). Some of the plates contained no colonies at all while many others were completely overgrown with fungi.

As was observed in the previous series the numbers show periodic rises and falls but there is no evidence that the general level rose or fell appreciably as a result of the water-logging.

In order to study the denitrifying properties of the organisms the more characteristic colonies appearing on the various plates were first developed in soil suspensions containing glucose (0.5 per cent.) and potassium nitrate (0.05 per cent.), then transferred into 100 gm. lots of the soils and incubated for three days at 35° and 20° C. Except for some slight reduction to nitrites no changes in the nitrate content could be observed at all.

The inconsistency of the counts combined with the tendency for increased development of fungi show that Giltay's Agar is not a suitable medium. Perhaps it is due to the fact that the concentrations of the

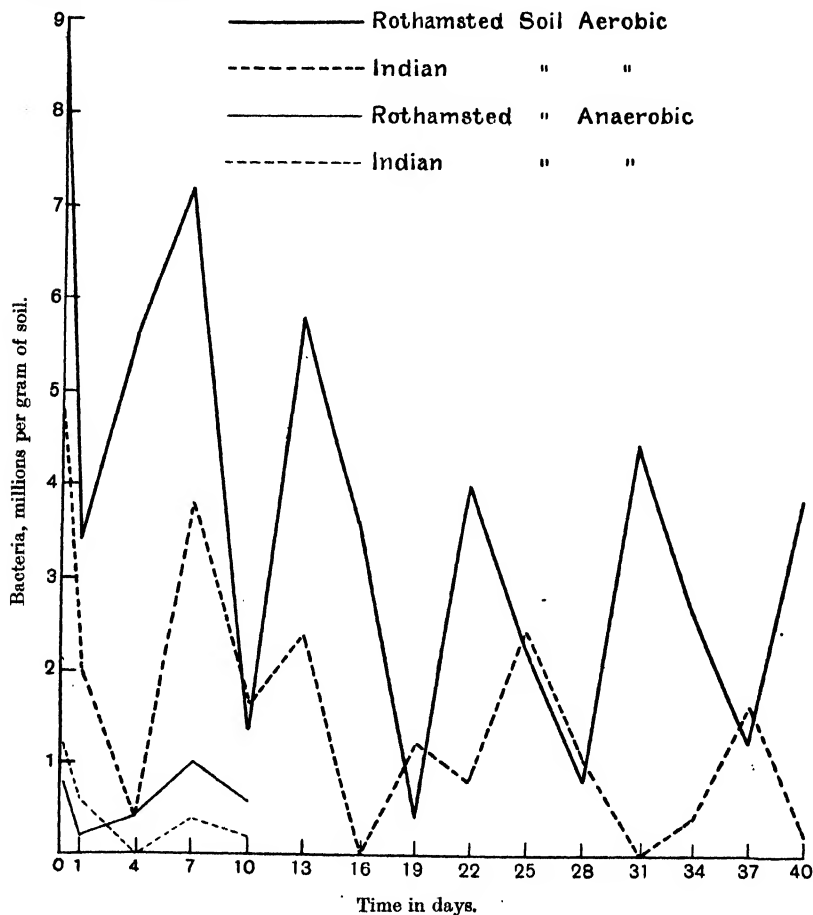


Graph 6. Variations in the bacterial counts, Thornton's Agar.



sugar and citric acid are too high as compared with the conditions normally prevailing in the soil.

The anaerobic platings were incubated in desiccators containing alkaline pyrogallol.



Graph 7. Bacterial counts on Giltay's Agar.

The numbers of colonies appearing after 10 days of incubation were very few, corresponding on the average to only one million per gram. (Graph 7.) None of them showed any nitrate-reducing power.

*Soil extract-gelatine.* Counts on soil extract-gelatine were obtained by plating on a medium composed of soil extract (sp. gr. 1.02) and gelatine (gold label, 18 per cent.) and incubating for five days at 20° C.

under aerobic and anaerobic conditions. The gelatine-liquefying colonies were also separately counted.

Under aerobic conditions there was an appreciable fall during the first four days in the total counts of the Rothamsted soil (20-13), whereas with the Indian soil the fall was not so marked. (Graph 8.) As observed on the other media the numbers then varied periodically. They remained however significantly lower than at the outset, so that there is evidence for a slight diminution as a result of the water-logging. There were very few colonies of fungi on the plates.

The counts of the gelatine-liquefiers also varied from time to time but they do not suggest any real variation. They kept more or less constant while the total counts decreased.

Very few colonies (about 1 M.P.G.) came out on the anaerobic plates. The numbers showed a slight decrease at the end of twenty-four hours and the counts during the later days were somewhat lower than at the beginning. The gelatine-liquefiers formed a large proportion of the total number and as observed under aerobic conditions their numbers showed no real change as a result of the water-logging.

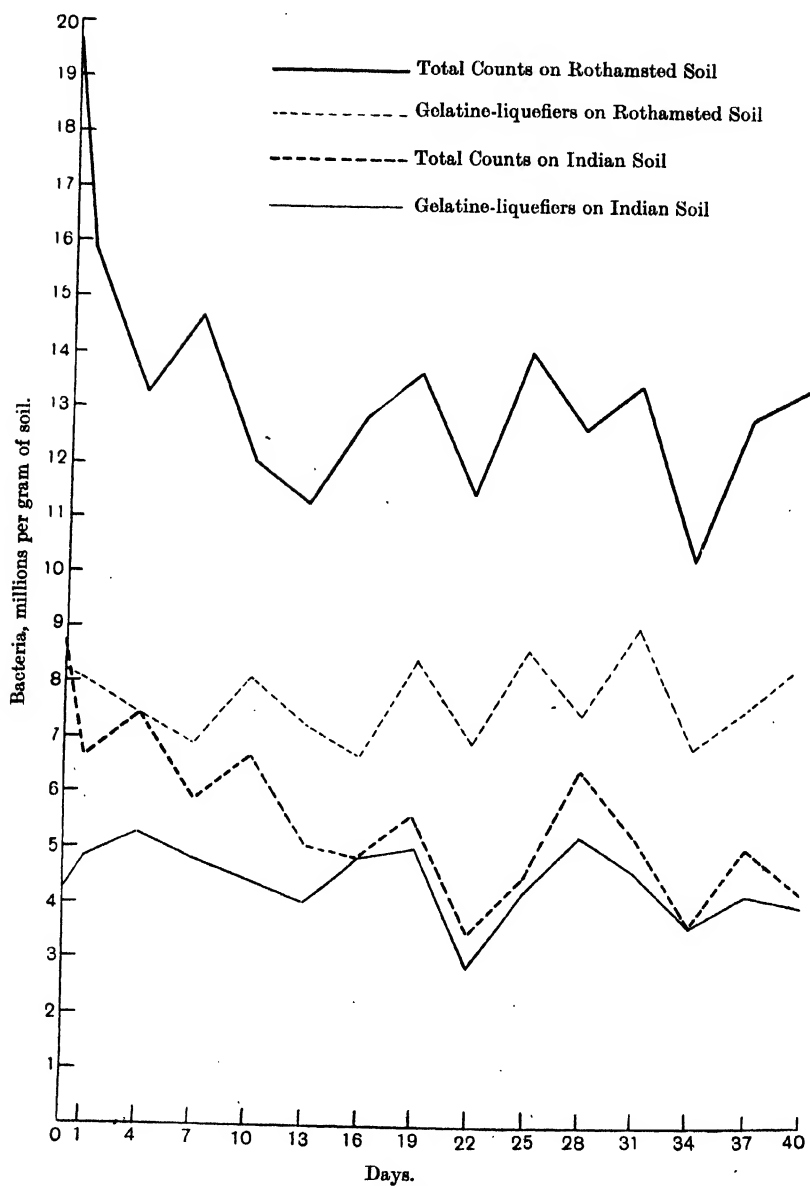
#### *The Nature of the Agency responsible for Ammonification in Water-logged Soils.*

Conn and Bright (2) have shown that when ammonification was brought about by biological activity there was a distinct increase in the numbers of certain non-spore-forming organisms which are active gelatine-liquefiers. If the increased ammonia production observed under the water-logged conditions had been brought about by the agency of such organisms we should expect to find an increase in the numbers of the gelatine-liquefiers. However no such increase took place, thus supporting the view that the reaction is probably not brought about by the activity of living organisms. The lack of appreciable carbon dioxide production also supports this view.

Russell and Hutchinson (23) have already noted that some ammonification takes place even where biological action is impossible and have suggested that it is probably brought about by enzyme action. A similar observation has been made by J. G. Lipman (13) who found that the quantity of ammonia produced from peptone was independent of the concentration of the latter. It is possible that in the present case we are dealing with a type of enzyme action.

The chemical mechanism of the reaction is apparently not one of oxidation. It is probably one of hydrolysis or reduction (1).

This question forms the subject of the next paper in this series.



Graph 8. Bacterial counts on Soil Extract-Gelatine (Aerobic).

## SUMMARY AND CONCLUSIONS.

Rothamsted and Indian soils were water-logged in the absence of freshly decomposing organic matter.

*Nitrogen changes:* Water-logging resulted in:

- (1) A distinct increase in the free and saline ammonia content.
- (2) A significant though only slight diminution in the nitric nitrogen.
- (3) No marked loss of ammonia by volatilisation or otherwise in the gaseous form: nor considerable variation in the nitrites: nor any observable diminution in the total nitrogen.

*Reaction:* Water-logging resulted in an increase in alkalinity; the increase in pH value was closely correlated with the corresponding increase in ammonia.

*Gaseous relations:* Water-logging resulted in:

- (1) No release of any soluble reducing matter capable of absorbing dissolved oxygen.
- (2) No appreciable carbon dioxide production.
- (3) An absorption of dissolved oxygen from the surface water. An equation has been worked out expressing the concentration gradient of dissolved oxygen with depth.

*Bacterial numbers:*

From bacterial counts on water-logged soils it was found that:

- (1) There was significant though slight decrease in bacterial numbers on Thornton's Agar.
- (2) Very few colonies were obtained by plating aerobically (and fewer still anaerobically) on Giltay's Agar. None of the organisms appearing on the plates brought about any nitrate reduction in soils.
- (3) The total counts on gelatine plates also showed some decrease. The numbers of gelatine-liquefiers on the other hand did not vary. There was no evidence to suggest that the increased production of ammonia was due to the activity of the gelatine-liquefiers.

*Agency responsible for ammonia formation:*

The results indicate that the formation of ammonia in water-logged soils is not due to biological action. It is suggested that the action is due to an enzyme.

In conclusion it is the pleasant duty of the author to acknowledge his indebtedness to Messrs H. J. Page and H. G. Thornton for their kind guidance and suggestive criticism.

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# BIOCHEMISTRY OF WATER-LOGGED SOILS.

## PART II.

### THE PRESENCE OF A DEAMINASE IN WATER-LOGGED SOILS AND ITS RÔLE IN THE PRODUCTION OF AMMONIA.

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(With Five Graphs.)

It has been shown in the previous paper that water-logging of soils led to increase in ammonia content; that the production of the latter involved no reduction of nitrates nor oxidation of proteins, nor increase in the bacterial numbers; and that the agency responsible for ammonia production is probably enzymic in nature. In the present paper an attempt is made to substantiate this hypothesis, in the case of soils free from freshly decomposing organic matter. The soils used were the same two as those used for the major part of the previous investigation, a Rothamsted soil (Barnfield) and an Indian soil (Coimbatore).

#### *The Distribution of Ammonia between the Soil and the Surface Water.*

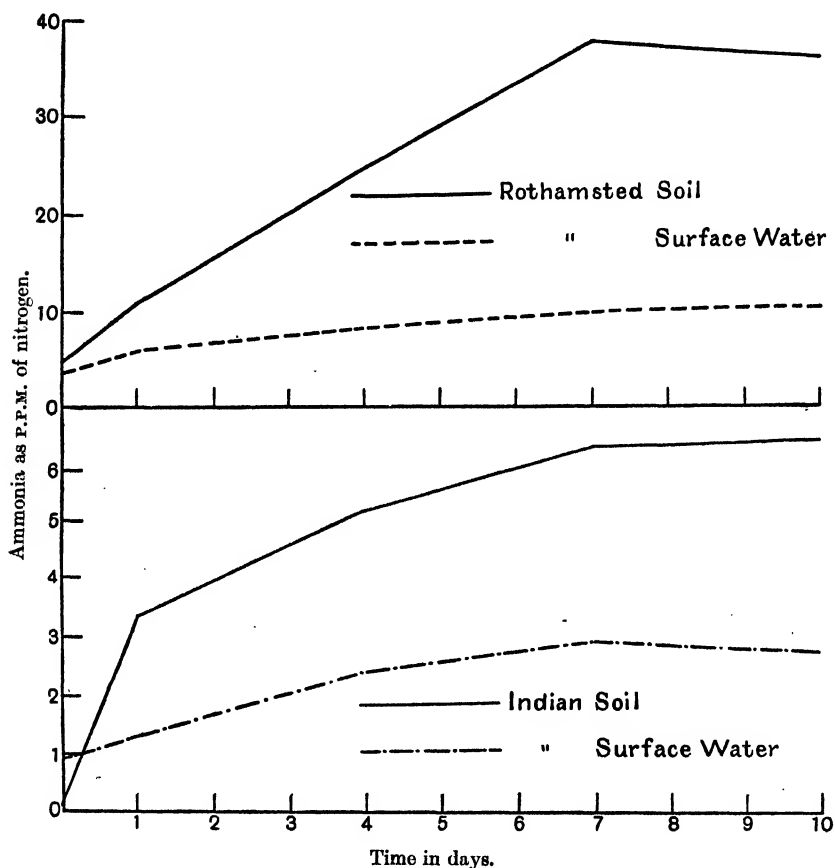
In order to obtain a better idea of the state of the ammonia formed, the amounts present at different times in the soil and the surface water were determined; the latter were estimated by filtering and distilling with magnesia, and the former by the McLean and Robinson (12) method.

It was found (see Graph 1) that though in both soils the quantities originally present in the surface water and the sediment were about the same, the soil ammonia soon showed a very distinct increase, whereas that present in the surface water changed only slightly. This difference was very pronounced in the Rothamsted sample, in which the amount

Table I. *Effect of Water-logging on the Ammonia Content of the Soil and of the Surface Water.*

Rothamsted soil					Indian soil			
Increase in soil			Increase in surface water		Increase in soil		Increase in surface water	
Time in days	Quantity as P.P.M.	As % of the total increase	Quantity as P.P.M.	As % of the total increase	Quantity as P.P.M.	As % of the total increase	Quantity as P.P.M.	As % of the total increase
1	6.2	72	2.4	28	3.25	89	0.4	11
4	19.9	82	4.5	18	5.35	78	1.5	22
7	33.0	84	6.3	16	6.05	75	2.0	25
10	31.1	82	6.9	18	6.15	77	1.8	23

present in the soil rose from 4.5 to 37.5 P.P.M. in seven days while that in the extract increased from 3.4 to only 9.7 P.P.M. In both soils the increase in soil ammonia constituted about 80 per cent. of the increase in total ammonia (Table I).



Graph 1. The distribution of ammonia between the soil sediment and the surface water.

In another experiment varying quantities of dilute ammonium hydroxide were added to water-logged soils and after half an hour the amounts in the soil and the surface water were determined. The results are shown in Table II.

It has already been observed on ordinary soils that although only about 30 per cent. of an added ammonium salt could be recovered by extraction with water, and added ammonium hydroxide is more difficult

to recover than ammonium sulphate, solutions of potassium or sodium chloride bring into solution practically all the ammonia originally present in or subsequently added to a soil(1, 6, 12). Moreover the extraction by the salt solution is practically instantaneous, as is the case with other exchangeable bases.

Table II. *Distribution of added Ammonia between the Soil and the Surface Water of Water-logged Soils.*

		Ammonia present, as P.P.M. of Nitrogen					
		Rothamsted soil			Indian soil		
No.	Quantity of added ammonia as P.P.M. of nitrogen	In the soil	In the surface water	Amt. of added $\text{NH}_3$ passing into surface water	In the soil	In the surface water	Amt. of added $\text{NH}_3$ in the surface water
I.	None	4.5	3.4	—	0.15	0.9	—
II.	20	21.6	6.3	2.9	16.3	4.8	3.9
III.	40	39.7	8.2	4.8	34.9	6.2	5.3
IV.	60	59.2	8.7	5.3	52.4	8.7	7.8
V.	80	78.5	9.4	6.0	71.0	10.1	9.2
VI.	100	98.1	9.8	6.4	90.3	10.8	9.9

Practically all the added ammonia passed into the soil and only a small part remained in the surface water.

Russell(16) has already suggested the possibility of a part of the soil ammonia being present in a state of stable chemical combination. McLean and Robinson(12) have shown that the exchangeable ammonia present in a soil at any given time is a definite quantity.

It appears that ammonia formed in or added to water-logged soils is held as an exchangeable base(10) in the same way as in ordinary soils. As soon as it is produced it is taken up by the soil colloids. Only a very small portion dissolves in the surface water and very little is lost by volatilisation. On extraction with salt solution, however, the ammonia is replaced and passes into the surface water from which it can be secured quantitatively by filtration and distillation with magnesia.

#### *The Nitrification of the Ammonia formed.*

Harrison and Iyer(7) have stated that nitrification proceeds rapidly when swamp soils are allowed to dry. In order to observe whether the ammonia formed during water-logging is similarly nitrified or is lost by volatilisation on drying, a series of determinations of the ammonia and nitrate contents were made on soils which were transferred to broad-bottomed conical flasks and allowed to undergo spontaneous evaporation at 35° C., after incubation for seven days with water. Samples were



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taken every seven days. The ammonia was determined by the McLean and Robinson method and the nitrate by the Devarda's Alloy method (18). The ammonia volatilising was estimated by absorption in acid (0.02N) and back titration against alkali.

Table III. *Ammonia lost by Volatilisation during the drying up of Water-logged Soils.*

Days	Ammonia lost from the Rothamsted soil	Ammonia lost from the Indian soil
7	1.3 P.P.M.	0.9 P.P.M.
14	1.9 "	1.3 "
21	1.8 "	1.2 "
28	2.1 "	1.0 "
Standard Error $\pm$ 0.3 P.P.M.		

The figures for the ammonia lost by volatilisation (Table III), show that only a very small portion of the total ammonia is thus accounted for. The total amounts lost are too minute to make much difference to the nitrogen content. During the drying, however, ammonia disappeared at a rapid rate. In the first three weeks the Rothamsted soil lost 42.7 P.P.M. and the Indian soil 6.3 P.P.M. of ammonia-nitrogen. During the final week, however, further reduction was not perceptible. The nitrate figures show that most of the ammonia had passed into that form (see Graph 2). The nitrate nitrogen increased from 7.5 to 36.8 P.P.M. in the Rothamsted soil and from 35.3 to 40.3 P.P.M. in the Indian soil, in three weeks.

Thus although no nitrification occurred as long as the soils remained water-logged, it commenced at a rapid rate when the soil dried and had free access to air.

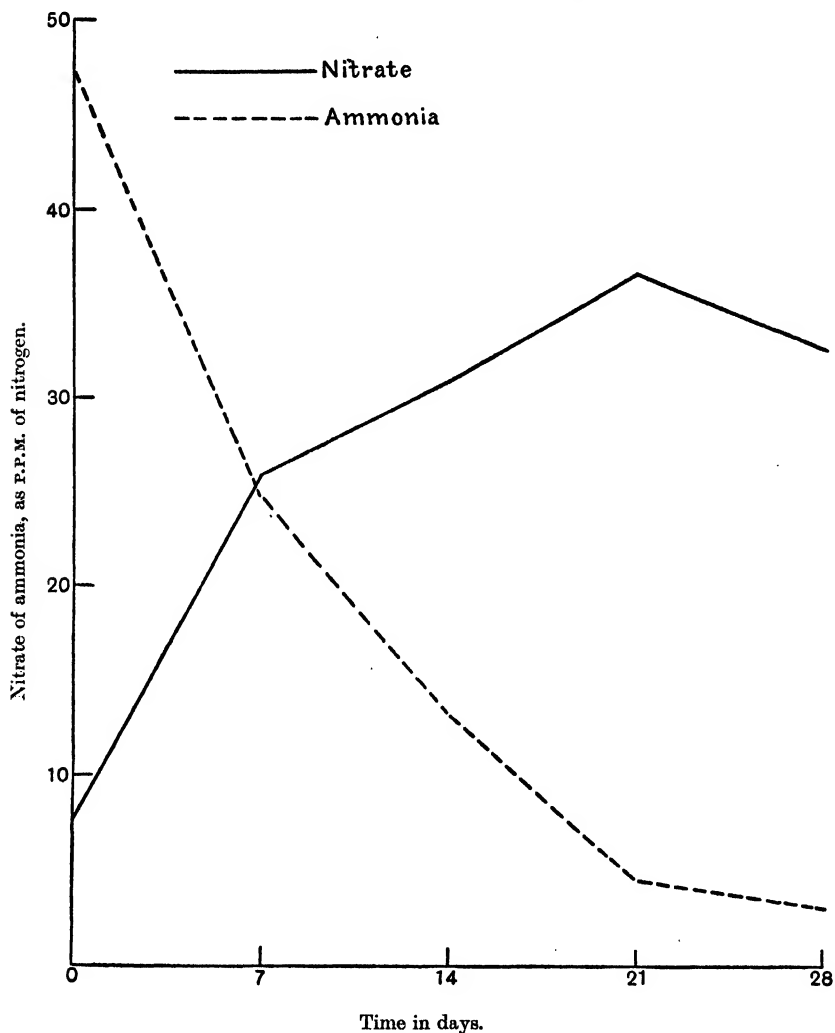
The positive correlation between the ammonia lost and the nitrate formed at the different times of observation is high for the Rothamsted soil, being + 0.91. It is not so high on the Indian soil, only + 0.59.

The differences between the ammonia lost and the corresponding amounts of nitrates formed may be taken to represent the quantities converted into other forms of nitrogen (Table IV).

Table IV. *Amounts of Ammoniacal Nitrogen lost during the drying up of Water-logged Soils and not accounted for as Nitrates or as Ammonia volatilised.*

Days	Rothamsted soil	Indian soil
7	+ 2.9 P.P.M.	- 2.3 P.P.M.
14	+ 8.7 "	- 2.5 "
21	+ 11.6 "	+ 0.1 "
28	+ 16.9 "	- 4.9 "

In spite of the close correlation between ammonia lost and nitrate formed in the Rothamsted soil, fairly large quantities of ammonia were converted into other forms by some unidentified agency.



Graph 2. Changes in the ammonia and nitrate content of water-logged Rothamsted soil when drying.

With the Indian soil, however, the nitrate formed, together with the ammonia lost by volatilisation, apparently exceeds the amount to be

accounted for. The differences are however not really significant. There is thus no evidence that, in that soil, ammonia passed into forms other than nitrate.

Godlewski(5) has shown that one of the essential requirements for active nitrification is free access to carbon dioxide, which the nitrifiers require for building their cells. This condition is not fulfilled in water-logged soil, in which practically all the ammonia exists in the soil sediment and the surface water contains practically no carbon dioxide.

#### *Can Ammonia be Produced in the Absence of Living Organisms?*

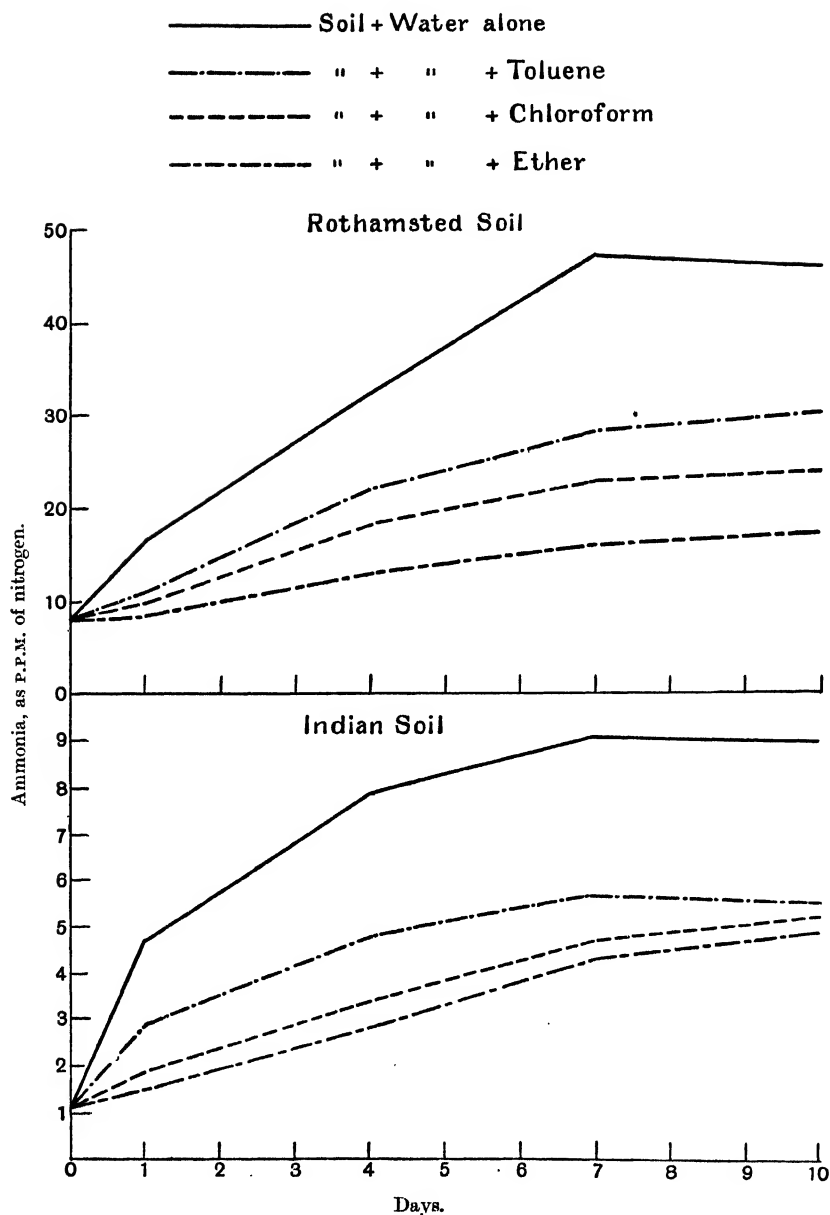
In their investigations on the partial sterilisation of soils Russell and Hutchinson(17) observed that a certain amount of ammonia was produced even in presence of volatile antiseptics. They suggested that it was probably due to enzyme action. The observations in the previous paper indicate that the ammonia produced under water-logged conditions cannot be due to biological respiration. In order therefore to ascertain whether the reaction can proceed in absence of living organisms and under conditions favourable to enzyme action water-logged soils saturated with volatile antiseptics were studied.

Preliminary trials showed that it was not possible to sterilise by adding volatile antiseptics to the soils already under water. Antiseptics only sparingly miscible with water either float on the surface or sink to the bottom leaving the soil almost unaffected. Platings on Thornton's medium(21) with incubation for three days showed that when the antiseptic was added (up to 20 per cent.) after the soil was water-logged bacterial colonies continued to appear and were sometimes more numerous than on controls without any antiseptic at all.

Attempts were therefore made to water-log the soils after allowing them to remain soaked under the antiseptics for twelve hours. 40 c.c. of the antiseptic were used for 100 gm. of soil and the mixtures were allowed to stand in a cool place, generally overnight, before the addition of water. The plates made from the soils so treated were almost entirely free from bacterial colonies, the two or three which appeared on some of the plates being probably due to accidental contamination during plating.

The two soils under investigation were thus treated with toluene, chloroform and ether and incubated, the Indian soil at 35° C. and the Rothamsted soil at 20° C., for 10 days.

Trials were also made with saturated solution of thymol as the antiseptic, but the results obtained were misleading because the thymol



Graph 3. Production of ammonia in presence of volatile antiseptics.

did not sterilise the soils. Even a direct microscopic examination of the soil showed it to be seething with rod-shaped organisms.

The results (Graph 3) show that the production of ammonia was quite significant in both the soils in presence of any of the three antiseptics. The quantities formed were never so high as in the untreated soils, but they followed about the same order in all the cases.

Toluene gave the highest figures and in both the soils there is a correspondence between the ammonia content of the untreated and the toluene-treated soils. On the 10th day, for instance, it is 45.9 P.P.M. in the former against 30.2 in the latter for the Rothamsted soil and 9.0 P.P.M. against 5.5 for the corresponding specimens of the Indian soil. The correlation coefficient between the values for the ammonia produced in the untreated and in the toluene-treated soils works out to + 0.9.

Quite similar results are obtained with the chloroform- and ether-treated specimens. They are slightly lower than those for the toluene-treated specimens, but bear out the same proportions to the figures for the untreated specimens.

Trials with antiseptics thus show conclusively that the production of ammonia can proceed in the absence of living organisms. Since under the experimental conditions purely chemical decomposition of protein or reduction of nitrates is not possible, it appears that the reaction is brought about by enzyme action.

As observed before, the non-production of carbon dioxide excludes reactions involving the oxidation of proteins. The constancy in the amount of dissolved oxygen in the surface water suggests that the production of ammonia was also not due to reduction. Hydrolysis suggests itself as the most plausible mechanism. It is necessary to determine the nature of the compounds hydrolysed before defining the character of the enzymes involved.

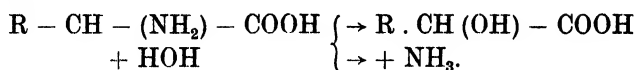
By eliminating the forms of nitrogen that are known not to be attacked it may be concluded that the ammonia is probably produced from proteins (or their derivatives) present in the soil.

The proteoclastic enzymes that are known to attack the more complex forms of proteins stop at the formation of the polypeptides and amino acids. They are not therefore the agents involved in the production of ammonia in water-logged soils.

The deaminases<sup>1</sup> however can directly hydrolyse amino acids and


<sup>1</sup> The terms "desamidase" and "deamidase" often found in the literature are not correct. It is an amino and not an amido group that is hydrolysed.

other protein derivatives to ammonia with the attendant formation of hydroxy acids.



If the deaminases are responsible for the production of ammonia this should be accompanied by formation of the corresponding hydroxy acids, and there should be no gas production. In order to ascertain whether proteins or their derivatives could be converted to ammonia in water-logged soils that had been treated with volatile antiseptics, a set of trials was carried out with a variety of amino bodies and vegetable and animal proteins. The soils in lots of 25 gm. were treated with toluene overnight as detailed previously. The amino bodies were added in 0.1 gm. lots with the water. The mixtures were incubated at 20° C. and 35° C. respectively for seven days and then analysed. The results obtained were as follow:

Table V. *Ammonia produced in Water-logged Soils, in the presence of Toluene, on the addition of various Nitrogen Compounds.*

No. of Expt.	Name and composition of the amino body added to the water-logged soils	Ammonia produced as P.P.M. of N	
		Rothamsted soil	Indian soil
I.	Nil	47.2	9.1
II.	Glycine, $\text{CH}_2(\text{NH}_2)\text{COOH}$	102.3	50.8
III.	Alanine, $\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$	53.1	14.3
IV.	Cysteine, $\text{CH}_2\text{SH}\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$	49.0	10.3
V.	Phenylglycine, $\text{C}_6\text{H}_5\cdot\text{CH}(\text{NH}_2)\text{COOH}$	46.0	10.7
VI.	Tyrosine, $\text{HO}\cdot\text{C}_6\text{H}_4\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$	48.1	11.0
VII.	Tryptophane, $\text{C}_8\text{H}_6\text{N}\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$	45.4	10.4
VIII.	Indole, 	48.7	9.8
IX.	Aspartic acid, $\text{CH}(\text{NH}_2)\cdot\text{COOH}$ $\text{CH}_2\cdot\text{COOH}$	58.3	18.2
X.	Asparagine, $\text{CH}(\text{NH}_2)\cdot\text{COOH}$ $\text{CH}_2\cdot\text{CO}\cdot\text{NH}_2$	62.5	19.6
XI.	Peptone (Witte's)	73.0	40.7
XII.	Gelatin (gold label)	51.3	12.4
XIII.	Glutenin (from wheat flour) <sup>1</sup>	46.5	10.7
XIV.	Legumin (from lentils) <sup>2</sup>	47.9	10.9

Standard Error =  $\pm 1.3$  P.P.M.

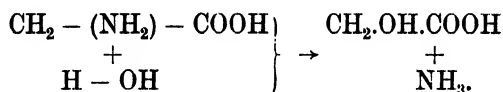
<sup>1</sup> Specially prepared by boiling wheat-gluten repeatedly with 80% alcohol, dissolving the residue in N/20 alkali and precipitating with dilute acetic acid—a pale yellow, amorphous powder, sparingly soluble in water (15).

<sup>2</sup> Prepared from lentil flour by extracting with 10% sodium chloride, boiling for a time to coagulate vicilin and saturating the cooled solution with ammonium sulphate. The legumin which was precipitated was taken up with excess of salt solution (10%) and precipitated by pouring into a large excess of water—a pale yellow, amorphous powder (14).

No gas production at all was observed from any of the soil samples, nor was any unusual odour observed except in the case of gelatin which emitted a somewhat putrid smell.

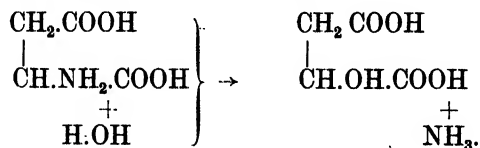
Appreciable amounts of ammonia were produced only from glycine, alanine (to a much smaller extent), aspartic acid, asparagine and peptone. The small increase with gelatin is scarcely significant. Practically none was produced from the other amino compounds.

*Glycine.* The quantity of ammonia produced was quite considerable being about 50 P.P.M. in each soil. To observe whether the corresponding hydroxy acid was produced, the aqueous extract of the soil was slightly acidified and shaken up with ether (in which glycine is insoluble) and the ether extract evaporated. The residue was extracted with a small quantity of water and heated with a few drops of concentrated sulphuric acid till the effervescence ceased. On addition of a drop of *p.* cresol to the mixture, on cooling, a green colour was obtained, indicating the presence of glycollic acid(3). The original reaction must therefore have been one of direct hydrolysis.



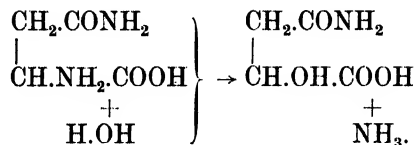
*Alanine.* The quantity of ammonia produced was so small as to suggest that it may have been due to small amounts of glycine present as an impurity. The ether extract did not give any tests for lactic acid. There is not therefore sufficient evidence to state that this amino acid underwent hydrolysis.

*Aspartic acid.* The amount of ammonia formed was quite appreciable. On addition of a solution of cobalt nitrate to the aqueous extract and boiling with excess of caustic soda a dark blue solution was obtained, indicating the presence of malic acid(21). The mechanism of the production of ammonia may be represented by



The quantity of ammonia formed from asparagine is not significantly different in either soil from that formed from aspartic acid. The amide group of asparagine does not seem to have caused any increase in am-

monia, indicating that probably it is not affected. The reaction therefrom seems to be:



*Peptone.* A considerable amount of ammonia (about 30 P.P.M.) was formed in both the soils from peptone. The medium turned distinctly acid to phenolphthalein and qualitative tests for glycollic and acetic acids gave positive results; no malic acid could be detected.

Levene and Van Slyke's analytical data<sup>(10)</sup> show that Witte's peptone contains practically all the amino acids tested in this work.

The presence of glycollic acid shows that the deaminase hydrolysed the glycine (0.8 per cent.) present. The absence of malic acid, even though peptone is stated to contain 1.7 per cent. of aspartic acid, and the presence of acetic acid, are not explained.

That the deaminising action is specific and not general in character is shown by the figures for the ammonia produced from the various compounds tested, and also by the selective preference shown among the amino acids in peptone. The presence of even the single methyl group in alanine makes it practically unhydrolysable although glycine is easily attacked. The other amino acids (except the aspartic acid and asparagine) are all more complex in configuration and the proteins very much more so. They are all unattacked, showing that the enzyme action is limited to the simplest amino acids.

Considering alanine, aspartic acid and asparagine as substituted glycines—

H — CH (NH <sub>2</sub> ) COOH	Glycine
CH <sub>3</sub> — CH (NH <sub>2</sub> ) COOH	Alanine (Methyl glycine)
HOOC.H <sub>2</sub> C — CH (NH <sub>2</sub> ).COOH	Aspartic Acid
H <sub>2</sub> NOC.H <sub>2</sub> C — CH (NH <sub>2</sub> ) COOH	Asparagine

—it is interesting to observe that the substitution of H by —CH<sub>2</sub>.COOH or CH<sub>2</sub>.CO.NH<sub>2</sub> hinders deamination less than its replacement by CH<sub>3</sub>.<sup>1</sup> The introduction of the other groups seems to make the amino acids unhydrolysable. The rate of production of ammonia from the amino acids is slow, for even with glycine, which underwent the largest con-

<sup>1</sup> Similar results have been obtained by Miss Bostock in her studies with emulsions of liver and intestinal mucosa (2), but whereas *in vivo* glycine is more rapidly deaminised than asparagine, *in vitro* exactly the reverse has been observed.



version, only 1/15th of the total possible amount was released in seven days. This may be due to a deleterious action of the antiseptic on the enzyme.

However, practically identical quantities of ammonia were finally produced in both the soils despite the difference in the temperatures of incubation. This suggests that the final reaction is probably independent of temperature. As with certain other enzymes, the quantities of the products formed in the end may be independent of the initial rate, which is determined by the temperature of incubation(4).

### *The Extraction of the Enzyme.*

In order to ascertain whether the enzyme could be extracted from the soils, 100 gm. lots of the two soils were extracted with a 5 per cent. aqueous solution of glycerol, saturated with toluene. The extracts were made up to 250 c.c. in each case and 25 c.c. used for each experiment. Glycine was used as the source of nitrogen and was added in 0.1 gm. lots for each trial. The mixtures were treated with more toluene and incubated for seven days at 20° and 35° respectively. The quantities of ammonia present at the end of the period are shown below:

Table VI. *Deamination of Glycine by Extracts of Water-logged Soils.*

No. of Expt.	Detail of treatment	Total quantity of Ammonia Nitrogen present	
		Extract from Rothamsted soil	Extract from Indian soil
I.	Glycerin extract alone + 10 c.c. toluene	0.134 mg.	0.071 mg.
II.	Glycerin extract boiled + 0.1 gm. glycine + 10 c.c. toluene	0.153 „	0.082 „
III.	Glycerin extract unboiled + 0.1 gm. glycine + 10 c.c. toluene	0.211 „	0.138 „

Standard Error =  $\pm 0.006$  mg.

The production of ammonia by treatment with the extract was thus quite marked for both soils. 0.077 mg. of nitrogen was released as ammonia by the extract from the Rothamsted soil and 0.067 mg. by that from the Indian soil. The difference between these quantities is not significant. Thus the deaminase is soluble and can be extracted. Since the extraction does not involve any application of pressure or rupturing of cells it must be considered to be an "exo" enzyme.

It is also seen that the enzyme is inactivated on boiling the extract. The slight apparent production of ammonia with the boiled extract is

not significant in the case of the Indian soil and barely so in the other.

Further proof of the independence of the reaction of temperature is provided by the fact that in the two trials the quantities of ammonia at the end of seven days were about the same though one set had been maintained at 20° and another at 35° C.

*The Isolation of an Active Preparation of the Enzyme and its Further Study.*

Since the enzyme is present in an easily extractable form an attempt was made to separate it from the soil with a view to obtaining more detailed information regarding its general characteristics.

Five kg. of the Rothamsted soil were first water-logged for seven days with five litres of water. 250 c.c. of glycerol were then added with sufficient toluene to saturate the mixture, which was well shaken and filtered. The extract was again treated with toluene and evaporated in shallow trays at 35–40° C. The residue was taken up with the minimum amount of water and the syrupy extract poured into 500 c.c. of absolute alcohol. A yellowish brown precipitate was obtained.

After standing overnight the alcohol was decanted off and the precipitate redissolved in the minimum amount of water. After reprecipitation in alcohol the yellowish product obtained was washed with alcohol and ether and dried at 35° C.

The final product (which weighed 0.18 gm.) was an orange-coloured amorphous substance which dissolved to a pale yellow solution in water.

Before proceeding further the preparation was tested for deaminising activity.

0.02 gm. was dissolved in 20 c.c. of water and 1 c.c. of the solution was added to 5 c.c. of a 1 per cent. solution of glycine with 2 c.c. of toluene and incubated for 10 days at 35° and 20° C. Samples were analysed at 3-day intervals.

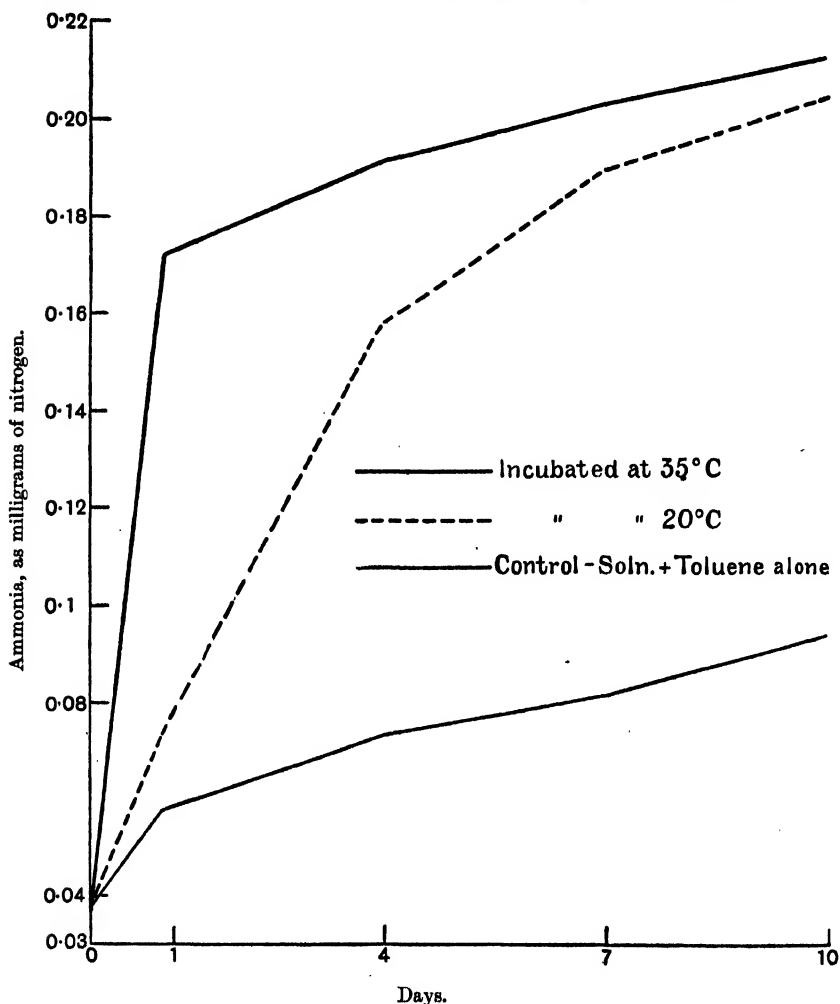
The results (Graph 4) show that the enzyme was present in the preparation.

At 35° C. the action was very rapid during the first 24 hours, about 0.14 mg. of the nitrogen from glycine being converted into ammonia. The rate fell off considerably soon after so that between the first and the fourth days only 0.019 mg. was converted—which is hardly significant. Later the curve becomes still more nearly horizontal showing that the activity was fast dying off.

At 20° C. the action was not so vigorous at the outset but continued more steadily. The rate however showed a slight slackening after the

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fourth day and more so after the seventh. The total quantities converted during corresponding periods appear to be less then at 35° C.; but the differences at the end of the seventh and tenth days are not significant.



Graph 4. Production of ammonia from glycine by the action of the deaminase.

The effect of temperature on the deaminisation now becomes obvious. At the higher temperature the reaction starts off very rapidly but soon slackens or dies out, at the lower it begins less vigorously but proceeds more steadily. After a certain time, whatever the temperature the total quantities reacted upon are about the same. In other words the quan-

tities finally converted are independent of the temperature of incubation.

The controls which contained only the enzyme solution with toluene also show quite appreciable increase in their ammonia contents. This may be due to the presence of deaminisable bodies in the preparation.

Further studies of the preparation were then made.

Its aqueous solution was neutral to litmus, but on the addition of formalin it developed acidity titratable to phenolphthalein. It contained carbon, hydrogen, oxygen and nitrogen, but no chlorine, sulphur, phosphorus or ash. Its composition, as determined by combustion and micro-Kjeldahl, was:

C	...	...	...	38.93 %
H	...	...	...	9.13 "
N	...	...	...	13.24 "
O	...	...	...	38.65 " (by difference).

Positive results were obtained with the biuret, xanthoproteic and Millon's tests, but there was no evidence for the presence of tryptophane. Molisch's reaction was also positive. The preparation therefore contained a protein-like body containing tyrosine and carbohydrate complexes.

No precipitate could be obtained on heating the solution either by itself or in presence of dilute acetic acid. It therefore seems probable that the preparation does not contain a true protein but rather a proteose or a peptone.

On saturating the aqueous solution with ammonium sulphate a dirty white precipitate was obtained showing that the substance cannot contain only a peptone. It is probably mainly of the nature of a proteose (9).

### *The Origin of the Deaminase in the Soil.*

Shibata<sup>(19)</sup> observed that permanent preparations of *Aspergillus Niger* showed deaminising action. In his study of the proteoclastic enzymes of soil fungi Waksman<sup>(22)</sup> noted that slight deaminisation attended the proteolysis of casein and peptone. The reaction was observed whether the organism had been grown on protein-containing or protein-free media.

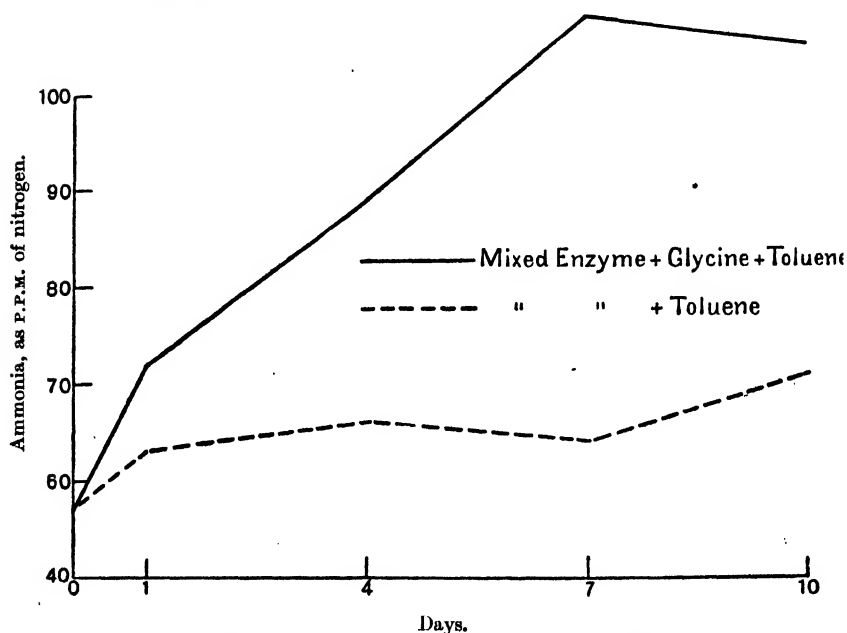
In order to obtain an idea of the deaminising action of the enzymes of the soil micro-organisms in general and thereby of their relation to the action exhibited by the soil as a whole a series of trials were carried out.

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Ten gm. of the Rothamsted soil were shaken vigorously with 250 c.c. of sterile saline<sup>1</sup> and 5 c.c. of the suspension inoculated into a culture solution composed<sup>2</sup> of

Sodium nitrate	...	...	...	...	0.25 gm.
Ammonium sulphate	...	...	...	...	0.20 "
Dipotassium phosphate ( $K_2HPO_4$ )	...	...	...	...	0.10 "
Magnesium sulphate	...	...	...	...	0.05 "
Ferric chloride	...	...	...	...	0.005 "
Calcium carbonate	...	...	...	...	2.0 "
Glucose	...	...	...	...	10.0 "
Water	...	...	...	...	500 c.c.

and incubated for seven days at 35° C. The mixed growth was then filtered through a Buchner funnel.



Graph 5. Deamination by the enzymes from the mixed fauna of the soil.

To 25 c.c. portions of filtrate glycine 0.01 gm. was added and incubated for 10 days in presence of toluene. A set of controls containing the extract and toluene alone were also maintained. Samples were analysed every three days for their ammonia content.

<sup>1</sup> Five gm. sodium chloride, 1 gm. magnesium sulphate, 1000 c.c. water.

<sup>2</sup> Protein substances were not included so as to avoid the deamination of the portions unused or only partially attacked; calcium carbonate was added to check the production of acidity.

The results (see Graph 5) show that the production of ammonia from glycine was quite significant. The reaction however was not a vigorous one. At the end of the fourth day the increase over the control (23 P.P.M.) was barely appreciable. On the 7th and 10th days it was a little more marked (44 and 34 P.P.M.) but is still small.

There is sufficient statistical evidence to state that the enzymes of the mixed flora of the soil can bring about deamination changes. It would be expected that a similar type of reaction could proceed more actively in the soil, which should present the most suitable medium for the enzymic activity of micro-organisms. It may therefore be concluded that the enzymes of the organisms of the soil are concerned at least in part in the deamination observed on water-logging.

### *The Rôle of the Deaminase in the Release of Plant Food.*

It has already been observed by Marchal<sup>(11)</sup> that the ammonification of proteins by *B. Mycoides* is never carried to a completion. Even with adequate proportions of available carbon to nitrogen, varying quantities of amino bodies are left behind unattacked. The work of Nencki<sup>(13)</sup> indicated that the lower amino acids, glycine and alanine, are highly resistant to biological oxidation or reduction. It would therefore appear that under ordinary soil conditions appreciable amounts of nitrogen would remain in forms in which they would not be very readily assimilated by plants<sup>(8)</sup>. This should be particularly so with the Rothamsted soil, which receives large quantities of organic manures (14 tons of dung to the acre) every year.

The presence of a deaminase in the soil however ensures that these amino bodies are converted into ammonia, which is then available for use as a plant nutrient, either with or without nitrification according to the conditions. This mode of enhancement of plant nutrition should be particularly prominent in the rice-growing swamp soils of the tropics, which generally have green manure (4000-5000 pounds per acre)<sup>1</sup> ploughed into them every year. The conditions there are more complicated because of the presence of decomposing organic matter, but the deaminising action ought to be still in progress and considerable amounts of easily assimilable nitrogen should thereby become available to the growing plant.

<sup>1</sup> Harrison and Iyer, *loc.cit.* p. 66.

## SUMMARY AND CONCLUSION.

(1) The ammonia formed in water-logged soils was present mostly in the soil sediment. The surface water contained only a very small portion of the total amount produced.

(2) Added ammonia was in a similar manner retained mostly in the soil itself. It could not be leached out by extraction with water nor volatilised with increase of temperature. There is evidence to show that the ammonia exists in the soil as an exchangeable base.

(3) On allowing the soils to dry out the ammonia disappeared rapidly and corresponding amounts of nitrates were formed. Very little ammonia was lost by volatilisation.

(4) The production of ammonia took place even in presence of volatile antiseptics. The reaction was shown to be brought about by a deaminase.

(5) Studies with a number of proteins and amino acids showed that only very simple amino compounds (glycine aspartic acid and asparagine) were deaminised. Witte's peptone, which contains amino acids, was also attacked.

(6) An active preparation of enzyme was extracted from the soil with an aqueous solution of glycerin saturated with toluene.

(7) Significant deaminising action was shown by the enzymes from cultures of the mixed microflora of the soils.

(8) By acting on amino bodies that are otherwise resistant to biological action the deaminase probably helps to release readily available plant food. Its action should be of great importance in tropical swamp soils.

In conclusion it is the pleasant duty of the author to acknowledge his indebtedness to Mr H. J. Page for his kind inspiration and suggestive criticism of the work.

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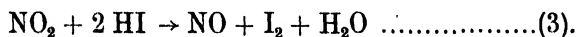
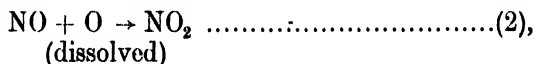
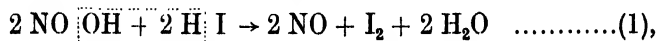
# AN IMPROVED METHOD FOR THE DETERMINATION OF DISSOLVED OXYGEN IN WATER.

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(With One Text-figure.)

THRESH's iodimetric method<sup>1</sup> is based on the ability of nitric oxide, even if present in traces, to catalyse the oxidation of hydriodic acid by the oxygen dissolved in the water and thereby liberate the equivalent amount of iodine. Thus, if excess of alkali iodide be left in a space free from atmospheric oxygen (by passing a continuous stream of coal gas through the apparatus) in presence of a small quantity of alkali nitrite, with a known volume of the water to be examined, the oxygen dissolved in the latter liberates its equivalent of iodine, which can be estimated by titration against standard thiosulphate. The reactions taking place may be represented by the following equations:



As will be seen from equation (1) the nitrite added also liberates its equivalent of iodine. When correction is made for this and for the oxygen dissolved in the reagents, the balance represents the iodine-equivalent of the oxygen dissolved in the water.

In the course of a comparative study of different methods for the estimation of dissolved oxygen arising out of an investigation of water-logged soil<sup>2</sup> the author noticed that the figures obtained by Thresh's method, for duplicate determinations, varied inversely with the time taken for the titration; the more the time taken, the less were the figures obtained. This is shown by the following figures obtained with Harpenden tap water at various times. The determinations were in duplicate

<sup>1</sup> *J.C.S.* 1890, **57**, p. 185.

<sup>2</sup> *Journ. Agr. Sci.* 1927, **17**, p. 429 *et seq.*

except that one set of figures was obtained by finishing the titration in 5 minutes and the other in 10 minutes.

No of Sample	Volume used c.c.	Temp. °C.	Pressure mm.	No of c.c. ·439 N/10 Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> required after correction		Oxygen as mg. per litre		Oxygen as c.c. at N.T.P.	
				Dup. I.	Dup. II.	Dup. I.	Dup. II.	Dup. I.	Dup. II.
				5 min.	10 min.	5 min.	10 min.	5 min.	10 min.
I.	889	3	747·8	35·2	32·7	13·91	12·92	9·74	9·04
II.	"	7	748·4	31·5	28·0	12·45	11·06	8·72	7·74
III.	"	13	749·2	25·7	22·6	10·15	8·93	7·10	6·25
IV.	"	18	748·9	22·6	19·4	8·93	7·67	6·25	5·37

The standard error of the mean of duplicates, calculated in the usual way from the above data, is found to be  $\pm 1·65$  c.c. Such a high standard error cannot be due to the method of titration because iodimetry is one of the most accurate of volumetric determinations and involves only very small errors. In a comparative set of titrations carried out between acidified iodine solution and thiosulphate the standard error of a set of five determinations was found to be  $\pm 0·035$  c.c. which is negligible.

Similar calculations for the standard errors for the duplicate determinations for "mg. per litre" and "c.c. per litre" at N.T.P. give values of  $\pm 0·65$  mg. and  $\pm 0·46$  c.c. respectively.

The differences in the time taken naturally suggest that some of the iodine formed is lost during the titration. In order to test this point, a set of titrations was carried out between acidified iodine and thiosulphate, varying periods being taken to complete the titration. A gentle current of coal gas was kept passing through the apparatus all the time and the contents frequently shaken to ensure even mixture. The figures obtained were as follows:

No. of Expt.	Temp. of Expt. °C.	Time taken for the Expt.	No. of c.c. of ·512 N/10 iodine solution taken	No. of c.c. of ·439 N/10 thiosulphate required		Mean
				Dup. I.	Dup. II.	
I.	16	Titrated at once. No gas passed through.	20 c.c.	23·3	23·3	23·3
II.	"	2 min.	"	22·5	22·4	22·45
III.	"	5 min.	"	21·4	21·2	21·3
IV.	"	10 min.	"	19·8	19·5	19·65
V.	"	15 min.	"	18·2	17·9	18·05

Standard error of mean of duplicates =  $\pm 0·13$  c.c.

A set of titrations was also carried out with the time constant and the speed of the gas passing through the apparatus varied. The figures obtained were as follows:

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No. of Expt.	Temp. of Expt. ° C.	Time taken for titn.	Speed of gas	No. of c.c. of $I_2$ soln. taken	No of c.c. of $\cdot 512 N/10$ $Na_2S_2O_3$ required	Mean titn. figs.
I.	18	5 min.	No gas passed	20 c.c.	23.3	23.3
II.	"	"	Just turned on	"	21.6	21.45
III.	"	"	Half turned on	"	20.2	20.05
IV.	"	"	Fully turned on	"	18.7	18.3

Standard error of mean of duplicates =  $\pm 0.18$  c.c.

The above figures are sufficient to show that the passage of gas through the apparatus during the titration involves the loss of iodine and that the greater the time taken the more iodine is lost. The gas current, however slow, involves some loss while speedier currents lead to a considerable loss.

Thresh<sup>1</sup> recognises the possibility of varying the results obtained by altering the speed of the gas; he recommends the passage of a gentle stream of gas, probably because it gives a more definite end-point than when no gas at all is passed. Evidently he did not reckon with the possibility of losing varying amounts of iodine even with a slow current of gas.

For any given concentration of iodine in solution there is a definite equilibrium concentration of iodine vapour in the gaseous phase above the liquid. When a continuous stream of gas is passed through the apparatus, this iodine vapour is swept away and lost, and, since the equilibrium between the liquid and gaseous phases is thus disturbed, more iodine passes into the gaseous phase and is in turn carried away. It is thus obvious that the longer the time that elapses before completion of the titration, or the more rapid the stream of gas, the greater will be the loss of iodine and the smaller the titration figure.

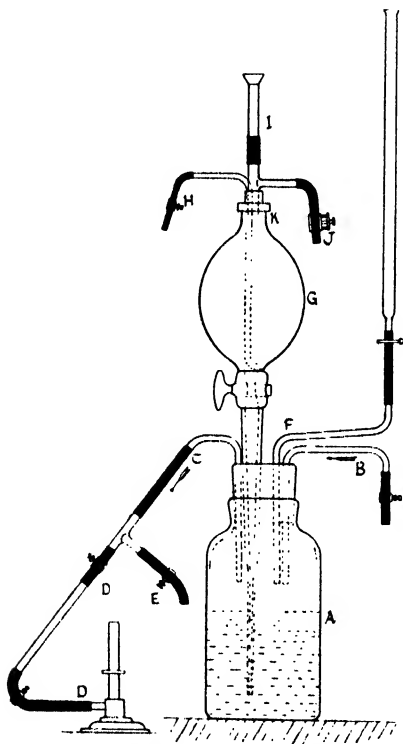
If the titration is carried out according to Thresh's directions, a slow current of gas passing through the apparatus, the end-point is bound to be sharp because the concentration of iodine in the space above is practically nil and even that could not dissolve back in the solution, the gas stream carrying it off from the sphere of action.

If, on the other hand, no gas is passed through the apparatus the iodine in the gaseous phase slowly redissolves in the solution, after an apparent end-point in the titration had been obtained, and the blue colour of starch iodide reappears. If more thiosulphate is added with each reappearance of the blue colour, the concentration of the iodine in the gaseous phase is finally reduced to a minimum. The process though tedious is the only way to obtain accurate titration-figures. These con-

<sup>1</sup> *Loc. cit.* p. 190.

siderations have led the author to devise a modification of Thresh's method which is capable of giving more accurate results.

The features of the apparatus, which is similar to that suggested by Thresh, can readily be seen from the accompanying figure. The large flat-bottomed jar *A* has a capacity of about  $1\frac{1}{2}$  litres. Coal gas enters through the tube *B* which is connected directly to a gas tap and dips a third of the way down into the flask *A*. The gas leaves through the tube *C* and



Sketch of the modified apparatus for the estimation of dissolved oxygen.

may pass either to an ordinary Bunsen burner<sup>1</sup> or through the rubber tube *E*. *G* is the flask (preferably a large separating funnel with a narrow neck and of capacity 750–1000 c.c.) in which the sample is collected and the liberation of the iodine completed. The bottom part of *G* is connected to a piece of glass tubing, if necessary, so that it dips right down to the bottom of *A*. This helps to let down the liquid from *G* very gently, avoiding unnecessary spurtng and spraying. The bent

<sup>1</sup> Burning of the gas through a burner is safer and much more convenient than burning in a jet at the end of a glass tube nozzle.

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tube *H* ends horizontally so that the liquid entering through it can do so very gently. *I* is a small funnel with a tap or pinch-cock at the bottom.

*Reagents required.* The same reagents as recommended by Thresh, viz.

- (1) Nitrite solution containing 5 per cent. nitrite and 20 per cent. KI;
- (2) 1 : 3  $\text{H}_2\text{SO}_4$  and  $N/10$  or  $N/20$   $\text{Na}_2\text{S}_2\text{O}_3$ .

### *The working of the apparatus.*

Before fitting the stopper to *A* introduce 1 c.c. of 5 per cent. starch solution into the flask; then make all the connections as shown in the figure and with the tap of *G* closed and the pinch-cock *E* open pass a fairly rapid current of coal gas through *A*. As soon as all the air has been driven out of *A*, the pinch-cock *E* is closed and the gas is burnt at the burner *D*. While the air from *A* is being steadily displaced the sample of water may be collected in *C* and the reactions allowed to commence.

The stopper from *G* is momentarily taken out and 2 c.c. of the nitrite-iodide solution introduced. The stopper is replaced, the pinch-cocks *H* and *J* are released while *I* is closed. *H* is connected to a tube dipping into the water to be analysed. *J* is connected to a pump and very gentle suction exerted. The rate of flow of the water can be controlled with the pinch-cock *J* so that no bubbles rise from the liquid to the surface; it should not exceed a few c.c. per minute. When the desired mark *K* in *G* is reached *G* and *H* are closed and the connections to the water source removed. The open space *K* should be as small as possible and the leading tubes should be semi-capillary so as to prevent much of the iodine and nitric oxide formed in *G* from diffusing into the space above.

Acid (1 : 3) is now poured into the funnel *I* up to the mark. Exactly 2 c.c. more of acid are then poured in. The pinch-cock on *I* is quickly released and the acid level allowed to drop down to the mark. This ensures that exactly 2 c.c. of acid is drawn in without, at the same time, drawing any air with it. The acid must be drawn rather quickly into *G* because otherwise the back pressure of nitric oxide inside the apparatus would prevent the whole flowing in. The whole apparatus is now well shaken so that the acid may react with all the nitrite and the nitric oxide resulting may be well distributed. After a quarter of an hour the passage of gas through *A* is stopped and the connecting tubes *B* and *D* are closed.

The side-tube *E* is now connected to the tube *J* and the pinch-cocks *E* and *J* are released. The tap of *G* is next opened and all the liquid is

allowed to flow gently into *A*. *E* is then momentarily connected also to *H* to release the water in it, *E* and *G* are then closed and the titration commenced.

The thiosulphate is added by drops with frequent shaking until the end-point is momentarily reached. A couple of minutes is next allowed for the nitric oxide to react with the oxygen of the reagent and more of the iodine in the gaseous phase to pass into solution. The blue colour reappears, and more thiosulphate is added until the colour again very nearly vanishes.

This addition of thiosulphate is repeated at short intervals until the final quantity required is less than two drops. The titration may now be stopped because the actual amount of iodine in the gaseous phase is very small. The best method of correcting for this small amount of iodine is to add .05 c.c. to the titration figure.

#### *Calculation of the amount of Dissolved Oxygen.*

Let us suppose that *V* is the volume of the water taken and *x* its oxygen equivalent in milligrams per c.c.

Similarly let *Y* represent the total oxygen equivalent of the prescribed quantities of nitrite, acid and starch solutions (*i.e.* 2 c.c., 2 c.c., and 1 c.c. respectively), and let *T* be the oxygen equivalent in mgm. per c.c. of the thiosulphate solution, of which *n* c.c. are used in the titration, and *Z* the dissolved oxygen equivalent of the same solution.

Then 
$$xV + Y + nZ = nT.$$

For the determination of *Y* and *Z* Thresh recommends separate determinations on the reagents alone, but the author finds the following method more reliable and convenient.

It is assumed that the dissolved oxygen *Z* per c.c. of the thiosulphate solution used is the same as that in the laboratory distilled water from which it is made.

Two dissolved oxygen determinations are made with the laboratory distilled water. In one the prescribed quantities of the reagents (*i.e.* 2 c.c., 2 c.c. and 1 c.c. respectively) and in the other half as much again (*i.e.* 3 c.c., 3 c.c. and 1.5 c.c. respectively), are used. Let *n* and *n'* be the two titration figures obtained, and let *V* and *V'* be the volumes of the water used.

Using the same notation as before,

$$\begin{aligned} Vx + Y + nZ &= nT, \\ V'x + 1.5Y + n'Z &= n'T. \end{aligned}$$

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Substituting  $x$  for  $Z$ ,

$$x [V + n] + Y = n \cdot T \quad \dots\dots\dots(1),$$

$$x [V' + n'] + 1.5Y = n \cdot T \quad \dots\dots\dots(2).$$

On solving for  $x$  and  $Y$  we have

$$x = \frac{T [3n - 2n']}{3 [V + n] - 2 [V' + n']}$$

and

$$Y = \frac{T \{n [V + n] - n [V' + n']\}}{3 [V + n] - 2 [V' + n']}.$$

As an illustration may be cited the figures obtained with the laboratory distilled water.

$$V = 889 \quad n = 13.50 \quad \text{Temp.} = 13^\circ \text{C.}$$

$$V' = 888 \quad n' = 14.35 \quad T = 0.7128 (0.891 N/10 \text{ Na}_2\text{S}_2\text{O}_3).$$

Substituting these values in the above equation we obtain

$$Z = X = 0.009317,$$

$$Y = 0.6080.$$

The following are the results obtained with Harpenden tap-water at various times. The water is somewhat hard with dissolved calcium bicarbonate but is entirely free from nitrites.

No. of Sample	Volume used c.c.	Temp. °C.	Pressure mm.	No. of c.c. of 0.439 N/10 Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> required		Oxygen as mg. per litre		Oxygen as c.c. per litre at N.T.P.	
				Dup. I.	Dup. II.	Dup. I.	Dup. II.	Dup. I.	Dup. II.
I.	889	6	728	32.05	31.90	10.96	10.90	7.67	7.63
II.	"	10	731.4	25.95	26.05	8.62	8.65	6.03	6.06
III.	"	14	745.9	22.85	22.70	7.42	7.36	5.19	5.15
IV.	"	17	746.1	21.45	21.35	6.88	6.84	4.82	4.79
$Z = 0.00932; Y = 0.6080; T = 0.3512.$									

The standard errors of the means of duplicate are:

For the titration figures,  $\pm 0.068$  c.c.

For the values of O<sub>2</sub> in mg. per litre,  $\pm 0.026$  mgm.

For the values of O<sub>2</sub> in c.c. per litre,  $\pm 0.019$  c.c. which compare favourably with the figures obtained on pages 469-70.

### *Correction for the nitrites present in the water.*

The nitrites present in most samples of water are too small to affect seriously the figures for dissolved oxygen. The correction to be made is generally small and when the quantity is under 10 mg. per litre may be conveniently determined by the Griess-Ilosway colorimetric method. The following are some of the figures obtained when varying quantities

of sodium nitrite were added to Harpenden tap-water and estimated by the Griess-Ilosway method.

No. of Expt.	Mg. of nitrite added per litre	Mg. per litre as obtained by Griess-Ilosway method	Correction to be applied for 889 c.c. water and .891 N/10 $\text{Na}_2\text{S}_2\text{O}_3$ (calci.) c.c.	Correction obtd. from colorimetric estimation c.c.	Error c.c.
I.	2	2.2	- 0.29	- 0.32	- 0.03
II.	4	4.1	- 0.58	- 0.59	- 0.01
III.	6	5.8	- 0.87	- 0.84	+ 0.03
IV.	8	8.3	- 1.16	- 1.30	- 0.04
V.	10	9.6	- 1.45	- 1.39	+ 0.06

As will be seen from the above data, the error involved in the correction is negligible.

The following are the figures obtained with distilled water saturated with air at various temperatures:

$$Z = 0.00932, \quad Y = 0.608, \quad T = 0.7128 \quad (0.891 \text{ N/10 } \text{Na}_2\text{S}_2\text{O}_3).$$

No. of Sample	Vol. used c.c.	Temp. ° C.	Pressure mm.	No. of c.c. of .891 N/10 $\text{Na}_2\text{S}_2\text{O}_3$ reqd.		Oxygen as mg. per litre		Oxygen as c.c. per litre at N.T.P.	
				Dup. I.	Dup. II.	Dup. I.	Dup. II.	Dup. I.	Dup. II.
I.	889	7	763.3	17.05	17.15	12.12	12.20	8.48	8.54
II.	"	10	763.8	16.30	16.25	11.53	11.50	8.07	8.05
III.	"	12	766.0	15.65	15.75	11.02	11.11	7.71	7.78
IV.	"	16	767.6	14.50	14.65	10.12	10.22	7.08	7.15

The standard error for the means of duplicate are:

For the titration figures,  $\pm 0.06$  c.c.

For the values of  $\text{O}_2$  in mg. per litre  $\pm 0.04$  mg.

For the values of  $\text{O}_2$  c.c. per litre  $\pm 0.03$  c.c.

The following are the figures obtained by previous workers<sup>1</sup> for dissolved oxygen in saturated distilled water at the same temperatures. It will be seen that most of the previous figures are somewhat low as compared with those obtained by the author.

The figures are all expressed as c.c. per litre at N.T.P.

Temp. ° C.	Bunsen	Roscoe and Lunt, dry	Roscoe and Lunt, wet	Winkler Dry	Dittmar	Thresh	Author
7	7.26	8.40	8.31	8.47	—	—	8.51
10	8.79	7.87	7.77	7.87	8.00	7.82	8.06
12	6.57	7.56	7.44	7.52	7.68	—	7.75
16	6.18	6.96	6.82	6.89	7.06	—	7.12

Bunsen's figures are evidently too low. The author's figures are comparable with those of Dittmar, the difference between them not being significant.

<sup>1</sup> Quoted from Adams, *J.C.S.* 1892, **61**, p. 322.



SUMMARY AND CONCLUSION.

The determination of dissolved oxygen by Thresh's method may lead to discordant results because of the loss of iodine, which is carried away by the gas passed through the apparatus during the titration.

A modified method is described, which eliminates the above error and obviates the necessity for making separate determinations of the dissolved oxygen of the reagents used.

Correction for the nitrites present in the water can be made by making separate determination by the Griess-Ilosway colorimetric method.

In conclusion, the author wishes to express his deep gratitude to Mr H. J. Page, for his suggestive criticism of the work.

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# THE NUTRITIVE AND MANURIAL VALUES OF SUGAR BEET TOPS.

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## INTRODUCTION.

THE heavy yields of sugar beet tops which remain on the land after the removal of the sugar beet crop may be utilised in two ways. They may either be ploughed into the land as manure, or they may be fed to stock. Where large areas of sugar beet are grown, and where in consequence it may not be possible to secure consumption of the whole of the tops before decomposition of the material sets in, a combination of these two methods of utilisation may be resorted to. In other words, the feeding of the tops may be continued so long as they remain wholesome, after which the remainder may be ploughed into the land.

In view of the present importance of the sugar beet crop in English agriculture, and the urgent necessity of making the fullest possible use of all the various by-products arising in connection with this crop both in the field and in the factory, it is of importance that data should be available relating to the value of sugar beet tops both as a feeding stuff and as a manure. The purpose of the present communication is to detail the results of investigations which have been carried out with a view to securing such information.

Certain aspects of these questions have already been discussed in earlier publications. The first of these<sup>(1)</sup> dealt mainly with the composition of sugar beet tops and the precautions which should be observed when feeding them to stock. Accounts were also given of the methods which are employed in Germany for the preservation of the tops either by artificial drying or by ensilage. In a second communication<sup>(2)</sup>, results obtained in investigations into the problem of ensiling sugar beet tops, alone or mixed with wet sugar beet pulp, were brought forward, and the feasibility of the adoption by the farmer of such a practice was critically discussed. It was shown that the continental practice of ensiling tops and wet pulp in alternate layers gives rise to a very palatable silage of high nutritive value, and that this method of preservation offers attractive possibilities on farms situated in the vicinity of beet sugar factories.

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### I. NUTRITIVE VALUE OF SUGAR BEET TOPS.

*General arrangement of digestion trial.* Since it was considered inadvisable to keep the sheep solely on a diet of sugar beet tops over a prolonged period, it was arranged that the feeding trial should include two separate periods of feeding. During the initial period, a daily ration consisting of 4000 gm. sugar beet tops and 600 gm. chaffed meadow hay was fed to the animals. To this ration was added 4 gm. precipitated chalk for the purpose of minimising any danger which might arise from the presence of oxalic acid in the beet leaves. The digestibility of the hay was determined in the second period, the daily ration in this instance being 1100 gm. of the chaffed meadow hay. The experimental periods, during which the excreta were collected and analysed, were of 14 days' duration.

The trial was carried out in duplicate, pure-bred Suffolk wether sheep, aged about 22 months, being employed for the purpose. The metabolism crates and the sheep harness, together with details of procedure in such experimental work, have been described in previous communications(3).

The tops which were used in the feeding trial formed part of the 1926 crop grown on the University Farm at Cambridge. The field from which they were taken is a light gravel of medium quality, overlying gault clay at a depth of five to six feet. The absolutely fresh tops were not fed to the sheep, but were allowed to wilt in thin layers on the stone floor of a cool room for periods varying from three to six days, the object being to secure by this means a reduction of the amount of oxalic acid in the beet leaves, and further to bring the tops into the condition in which they would be fed to stock in farming practice. It was found in further tests that under such conditions of wilting no decay appeared even after the lapse of a month, excepting traces on the cut surface of the root. After 14 days' wilting, the tops had a pleasant smell similar to that of good hay.

Prior to weighing out the rations, the tops were carefully freed from adhering soil and then cut up in a large mechanical meat mincer provided with revolving knives. From the thoroughly mixed mass, the rations for the day were weighed out, and at the same time samples were also taken for analytical purposes. Moisture determinations on the tops as consumed by the sheep were carried out daily.

*Palatability of sugar beet tops.* The sheep remained in good condition throughout the trial. They consumed the mixed ration with evident relish, displaying a particular fondness for the tops. Indeed, such small food residues as were left in this period consisted entirely of hay.

Table I. *Average Composition of Sugar Beet Tops, Meadow Hay and Composite Samples of Faeces. (On basis of dry matter.)*

	Sugar beet tops %	Meadow hay %	Faeces			
			Sheep I		Sheep II	
			Period 1 %	Period 2 %	Period 1 %	Period 2 %
Crude protein	12.55	8.65	11.15	9.88	10.73	9.54
Ether extract	2.76	3.52	3.11	2.94	3.11	2.84
N-free extractives	53.61	50.12	37.76	48.44	36.80	48.48
Crude fibre	9.92	30.10	16.30	26.85	16.51	27.20
Ash	21.16	7.61	31.68	11.89	32.85	11.94
True protein	8.76	7.24	9.17	8.23	9.34	8.10
"Amides"	3.79	1.41	1.98	1.65	1.39	1.44
Pepsin-HCl soluble protein	8.94	—	—	—	—	—
Total phosphate ( $P_2O_5$ )	0.672	0.488	1.490	1.205	1.642	1.238
Lime ( $CaO$ )	1.320	0.815	3.640	1.863	3.474	1.878
Silica	7.73	4.76	22.51	7.43	23.15	7.28

Mean dry matter in hay as consumed =  $\begin{cases} 86.49\% \text{ (Period 1).} \\ 87.64\% \text{ (Period 2).} \end{cases}$

Mean dry matter in tops as consumed = 21.68 %.

	Sheep I		Sheep II	
	Period 1 gm.	Period 2 gm.	Period 1 gm.	Period 2 gm.
Net daily dry matter in hay consumed*	515.1	962.6	516.9	961.0
Mean weight of faeces voided per day	1165.0	830.0	1157.0	808.0
Mean dry matter in faeces per day	472.1	354.4	449.3	344.5

\*Allowing for small amounts of hay residues.

Table II. *Digestibility of Meadow Hay. (Period 2.)*

Daily ration: 1100 gm. chaffed meadow hay.

	Sheep I						
	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Consumed	962.60	889.35	83.27	33.88	482.46	289.74	73.25
Voided	354.40	312.26	37.69*	10.42	171.67	95.16	42.14
Digested	608.20	577.09	45.58	23.46	310.79	194.58	31.11
Dig. coefficients %	63.18	64.89	54.74	69.25	64.42	67.16	42.47

	Sheep II						
	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Consumed	961.00	887.87	83.13	33.83	481.65	289.26	73.13
Voided	344.50	303.37	35.13*	9.78	167.01	93.70	41.13
Digested	616.50	584.50	48.00	24.05	314.64	195.56	32.00
Dig. coefficients %	64.15	65.83	57.74	71.09	65.33	67.61	43.76

\* Calculated on nitrogen as determined in fresh faeces.

*Ratio of crown to leaf in sugar beet tops.* Since the composition and feeding value of sugar beet tops will be influenced to an important degree by the ratio of crown to leaf in the sample, it was considered essential to secure information on this point during the carrying out of the present

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trials. On every day during the period of 14 days, 40 tops were divided into crown and leaf and the total weights of crown and leaf were determined separately. It was found that one-quarter of the tops consisted of crown and three-quarters of leaf, this rather high proportion of crown being due to the fact that the leaves were somewhat stunted in growth, whereas the roots were of medium size.

Table III. *Digestibility of Sugar Beet Tops. (Period 1.)*

Daily ration { 4000 gm. sugar beet tops, 600 gm. chaffed meadow hay, 4 gm. precipitated chalk.							
<i>Sheep I</i>							
	Dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Consumed:							
Tops	867.20	683.70	108.83	23.93	464.91	86.03	183.50
Hay	515.10	475.90	44.56	18.13	258.17	155.04	39.20
Chalk	4.00	—	—	—	—	—	4.00
Total	1386.30	1159.60	153.39	42.06	723.08	241.07	226.70
Voided	472.10	322.54	53.50*	14.68	178.26	76.95	149.56
Digested:							
Total	914.20	837.06	99.89	27.38	544.82	164.12	77.14
From hay	325.40	308.81	24.39	12.56	166.31	104.12	16.65
From tops							
+ chalk	588.80	528.25	75.50	14.82	378.51	60.00	60.49
Dig. coefficients of tops %	67.89	77.26	69.38	61.93	81.42	69.74	32.97
<i>Sheep II</i>							
Consumed:							
Tops	867.20	683.70	108.83	23.93	464.91	86.03	183.50
Hay	516.90	477.56	44.71	18.19	259.07	155.59	39.34
Chalk	4.00	—	—	—	—	—	4.00
Total	1388.10	1161.26	153.54	42.12	723.98	241.62	226.84
Voided	449.30	301.70	50.38*	13.97	165.34	74.18	147.60
Digested:							
Total	938.80	859.56	103.16	28.15	558.64	167.44	79.24
From hay	331.60	314.38	25.82	12.93	169.25	105.19	17.22
From tops							
+ chalk	607.20	545.18	77.34	15.22	389.39	62.25	62.02
Dig. coefficients of tops %	70.02	79.74	71.07	63.60	83.75	72.36	33.80
Mean dig. coefficients %	69.0	78.5	70.2†	62.8	82.6	71.1	33.4

\* Calculated on nitrogen as determined on fresh faeces.

† Digestion coefficient of crude protein as determined by pepsin = HCl = 71.2 %.

The sugar content of the crowns, determined by the usual saccharimetric method employed for the roots, was found to be as high as 17.6 per cent. Assuming the amount of sugar in the leaves to be small, it

follows that the tops contained 4.4 per cent. of sugar, this representing roughly one-fifth of the total dry matter in the tops as fed to the sheep in the digestion trials.

Since the percentage of sugar, as also of protein, is determined by the ratio of leaf to crown in the sample, it follows that a high protein content will usually be associated with a low content of sugar. Thus, in the analysis of another sample of tops from a different crop of sugar beet, the sugar content was found to be only 12.55 per cent. of the dry matter, whereas the percentage of crude protein was as high as 17.52 per cent. (as compared with the value 12.55 per cent. for the tops used in the feeding trial). In numerous German analyses of sugar beet tops, Honcamp and Katayama<sup>(4)</sup> obtained results for the percentage of sugar in the dry matter ranging from 14 to 23 per cent.

*Comments on Table II.* The only feature requiring special comment is the somewhat surprising result that, notwithstanding the indifferent quality of the hay as revealed by analysis, both sheep were able to digest the fibre in the hay to a slightly greater extent than the carbohydrate. Careful repetitions of the analytical procedure on the samples of hay and faeces showed that this finding was not connected in any way with possible errors of experiment.

*Comments on Table III.* The mean digestion coefficients obtained in the present trial are compared in Table IV with corresponding figures given by Kellner<sup>(5)</sup>. The digestion coefficients for the dry matter of the tops are omitted, since owing to the high content of ash, partly composed of soil, in the tops, these figures must be regarded as possessing little or no significance.

Table IV. *Digestion Coefficients of Constituents in Sugar Beet Tops.*

	Present investigation %	Kellner (mean of 4 investigations) %
Organic matter	78.5	77.0
Crude protein	70.2	74.0
Ether extract	62.8	55.0
N-free extractives	82.6	80.0
Crude fibre	71.1	70.0

The two series of results recorded in Table IV display satisfactory agreement, the only marked divergence being in the case of the digestion coefficients of the ether-soluble constituent. This, however, is not serious, in view of the small amount of oil contained in sugar beet tops. In a recent German trial, Honcamp and his co-workers<sup>(6)</sup> obtained the

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value 78.5 per cent. for the digestion coefficient of the organic matter of sugar beet tops, a figure in perfect agreement with the results of the present experiment.

It may be concluded that sugar beet tops form a feeding stuff of high digestibility, the carbohydrate constituent, consisting partly of sugar derived from the crowns, being especially digestible. The high fibre digestion coefficient also constitutes a noteworthy feature.

A further comparison is shown in Table V, where the data for crude and digestible composition of the tops used in the present trial are recorded, together with corresponding data given by Kellner. For the purposes of the comparison, the two sets of values are expressed on the basis of the mean water content of the German samples, namely 83.8 per cent., since the higher dry matter content of the English sample was due to the fact that the tops had been allowed to wilt under cover and not under the usual field conditions.

Table V. *Crude and Digestible Constituents in Sugar Beet Tops.*

	Crude constituents		Digestible constituents	
	Present investigation	Kellner	Present investigation	Kellner
	%	%	%	%
Moisture	83.80	83.8	83.80	83.8
Crude protein	2.03	2.3	1.43	1.7
Ether extract	0.45	0.4	0.28	0.2
N-free extractives	8.68	7.4	7.17	5.9
Crude fibre	1.61	1.6	1.14	1.1
Ash	3.43	4.8	—	—
Total organic matter	12.77	11.7	10.02	8.9
Nutritive ratio	—	—	6.3	4.4
Starch equivalent per 100 lb.	—	—	8.55*	7.5*

\*  $V = 84\%$ .

The comparative data given in Table V show that the sugar beet tops employed in the present investigation were richer in organic matter and carbohydrate, and poorer in ash, than the German sample. The probable reasons for these differences are: (1) The tops were carefully freed from adhering soil before being sampled for analysis. (2) The proportion of crown to leaf was probably higher than in the German sample. These circumstances also account for the higher percentage of digestible organic matter, the higher starch equivalent and the wider nutritive ratio which characterised the English sample.

If the values be calculated to the basis of dry matter, it is seen that 100 lb. of the dry matter of the tops contained 61.8 lb. digestible organic matter, including 52.8 lb. starch equivalent, 8.8 lb. dig. protein, 1.7 lb.

dig. ether extract, 44.2 lb. dig. carbohydrate and 7 lb. dig. fibre. A more concrete idea of the nutritive value of sugar beet tops may be gained from the data given in Table VI, where it is shown that 40 lb. of mangolds (10.7 per cent. dry matter) may be replaced in the rations of stock by 25 lb. of sugar beet tops (16.2 per cent. dry matter).

Table VI. *Replaceability of Mangolds by Sugar Beet Tops.*

	lb. dry matter	lb. starch equivalent	lb. digestible protein
40 lb. mangolds	4.30	2.20	0.28
25 lb. sugar beet tops	4.05	2.14	0.36

Table VII. *Showing Weights of Sheep and Nitrogen, Phosphate and Lime Balances in the Sheep during the Digestion Trials.*

Period	Con- stituent	Sheep	Consumed per day			Voided per day			Mean daily balance gm.
			Hay gm.	Tops + chalk gm.	Total gm.	Faeces gm.	Urino gm.	Total gm.	
Meadow hay	N	I	13.32	—	13.32	6.03	5.11	11.14	+2.18
		II	13.30	—	13.30	5.62	7.09	12.71	+0.59
	P <sub>2</sub> O <sub>5</sub>	I	4.70	—	4.70	4.27	0.02	4.29	+0.41
		II	4.69	—	4.69	4.27	0.03	4.30	+0.39
	CaO	I	7.85	—	7.85	6.60	0.06	6.66	+1.19
		II	7.83	—	7.83	6.47	0.07	6.54	+1.29
Hay + tops + chalk	N	I	7.13	17.41	24.54	8.56	11.01	19.57	+4.97
		II	7.16	17.41	24.57	8.06	12.98	21.04	+3.53
	P <sub>2</sub> O <sub>5</sub>	I	2.51	5.83	8.34	7.03	0.16	7.19	+1.15
		II	2.52	5.83	8.35	7.38	0.08	7.46	+0.89
	CaO	I	4.20	13.69	17.89	16.71	0.14	16.85	+1.04
		II	4.21	13.69	17.90	15.61	0.20	15.81	+2.09

Period	Date (1926)	Weights of sheep			
		I		II	
		st.	lb.	st.	lb.
Hay + tops + chalk	Nov. 16	9	8	9	3
	Dec. 2	9	11	9	1
Meadow hay	Dec. 7	10	0	9	6
	Dec. 23	10	0	9	5

*Comments on Table VII.* The results for the lime balances in the sheep constitute a significant feature of this set of data. That the animals were still able to store lime is shown by the fact that on the hay ration containing rather less than 8 gm. CaO, the mean daily balance for both sheep was +1.24 gm. Although the daily consumption of lime in the sugar beet tops period rose to nearly 18 gm., there was no corresponding sharp rise in the lime balances, the mean value for both sheep in this



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period being about 1.6 gm. CaO per day. This result suggests that the lime in the sugar beet tops may not be in a form available to the animal, possibly on account of the presence of oxalic acid in the leaves. That the latter are rich in oxalic acid is shown by the results of German analyses (4), which have demonstrated that this undesirable constituent may constitute as much as 6 per cent. of the dry matter of the unwilted leaves. It is possible, therefore, although the final proof of the assertion must be left to a further investigation, that the oxalic acid in the beet leaves may render not only the lime of the tops unavailable, but also more or less of the lime in other feeding stuffs which may be fed along with the tops. Obviously this form of lime starvation might have undesirable consequences when the tops are being fed to the dairy herd or the ewe flock, and it would appear desirable under such circumstances to allow the animals access to lime compounds in some form or other. The writers hope to be able to enquire more fully into this aspect of the question during the coming season.

### II. THE MANURIAL VALUE OF SUGAR BEET TOPS.

*Objects and procedure.* Enquiries have been received from different sources as to the value which should be assigned to sugar beet tops when they are ploughed into the land as a manure. With the object of securing reliable information on this question, samples of tops from the 1926 sugar beet crop were collected from a number of farms distributed over the Eastern Counties. The samples were divided, for comparative purposes, into four broad groups, according to the type of soil (light, medium, heavy or fen) on which they had been grown.

The samples of tops were received over a period of about two months, beginning October 8, 1926. Every sample was finely cut up in a mechanical meat mincer fitted with revolving knives. The minced material was well mixed, and representative samples of 200 gm. were dried down for the moisture determination. The dried residues were finely ground up and, after air-drying, were utilised for analysis.

The three constituents to be estimated for the calculation of manurial value are nitrogen, phosphoric acid and potash. In view of the small amount of extra labour involved, it was decided to secure further data in respect of the content of ash, silica, soda and lime. Nitrogen was estimated by the Kjeldahl method, phosphoric acid by the Pemberton-Neumann method, and lime by the precipitation of calcium oxalate from the ash extract by means of saturated ammonium oxalate in the presence

of acetic acid. Potash and soda were determined by the modified cobaltinitrite method in use at the Rowett Research Institute.

Table VIII. *Showing Average Nitrogen and Mineral Content of Sugar Beet Tops from Different Types of Soils.* (Percentage expressed on dry matter basis.)

Soil type	No. of samples	Dry matter* %	Total ash %	SiO <sub>2</sub> %	N %	P <sub>2</sub> O <sub>5</sub> %	CaO %	K <sub>2</sub> O %	Na <sub>2</sub> O %
Light	10	17.7	19.63	5.22	2.00	0.681	1.86	3.56	2.70
Medium	7	16.8	19.16	5.57	1.85	0.568	2.31	3.07	2.78
Heavy	2	15.9	17.49	1.61	2.12	0.555	3.65	3.53	2.06
Fen	6	12.9	16.21	0.52	2.38	0.492	1.84	3.34	3.15

\* Showing mean percentage of dry matter in samples as received.

*Comments on Table VIII.* It is clear from the detailed figures recorded in the Appendix to this section that, on account of the considerable variation in the mineral composition of the samples of sugar beet tops included in any one group, care must be exercised in attempting to discover correlations between mineral composition and soil type. The only conclusion in this regard which can be made with certainty is that fen-grown tops contain a much smaller percentage of silica than tops grown on the other types of soil. It would appear however that the mineral composition of the tops is more affected by chemical factors such as manurial treatment, soil reaction and so forth than by the mere physical characters of the soil.

It will be noted that sugar beet tops are extremely rich in inorganic constituents, the percentage of total ash in the individual samples ranging from 13.7 to 26.0 per cent. of the dry matter, with an average value of 18.5 per cent. The results for one or two samples, where the silica content was unusually high, would suggest that in these cases a little soil may have found its way into the analytical sample. The samples for the most part, however, arrived in a very clean condition, and care was exercised to remove any soil which might be seen adhering to the leaves. It may therefore be concluded that the high ash content of the tops is independent of earthy impurity, especially as the high value of 21.2 per cent. was obtained for the sample of tops analysed in connection with the feeding trial, and in this case great care was taken to ensure the complete removal of soil before proceeding with sampling and analysis. The mean percentages of silica-free ash in the samples from the heavy and fen soils show striking agreement, being 15.9 and 15.7 per cent. of the dry matter respectively. The corresponding values for the light and medium soil samples of tops were 14.4 and 13.6 per cent.

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Other general features which are brought out consistently by the analytical data are (1) the high percentages of potash, soda and lime in the tops and (2) the relatively low percentage of phosphoric acid. These facts are emphasised in Table IX, where the average composition of the silica-free ash in the sugar beet tops is also compared with corresponding data for mangold leaves(7).

Table IX. *Mineral Composition of Sugar Beet Tops and Mangold Leaves.*  
(Results expressed as percentages of silica-free ash.)

	Sugar beet tops %	Mangold leaves %
Lime (CaO)	14.5	11.0
Phosphoric acid ( $P_2O_5$ )	4.0	6.7
Potash ( $K_2O$ )	23.2	31.8
Soda ( $Na_2O$ )	19.0	20.1

It is clear from Table IX that mangold leaves display the same characteristics in respect of mineral composition as do sugar beet tops. The proportion of phosphoric acid is low, whereas lime, soda and potash, particularly the last named, are present in large amount. The high percentage of soda is of interest, explaining why the mangold crop responds successfully to dressings of common salt and nitrate of soda and suggesting that, under certain circumstances, lack of sodium compounds might constitute the limiting factor in the growth of the sugar beet crop.

*Calculation of manurial value of sugar beet tops.* The necessary data for calculating the manurial value of a ton of fresh tops are given in Table X. Owing to the low dry matter content which characterises the very heavy yields of tops obtained on fen soils, it is advisable to adopt a separate standard, as shown in Table X, in assessing their manurial value.

Table X. *Manurial Constituents in Fresh Sugar Beet Tops.*

	Average values (excluding fen tops) (% dry matter = 17.2)	Fen-grown tops (% dry matter = 12.9)
Nitrogen	0.34	0.31
$P_2O_5$	0.11	0.06
$K_2O$	0.58	0.43

If the tops are ploughed into the land, it may be assumed that the whole of the nitrogen, phosphate and potash is available as manure. When stock are allowed to consume them off the land, then it is assumed that only half the nitrogen goes into the excreta and three-quarters each

of the phosphate and potash. The current prices per unit of the manurial constituents should be used for calculating the manurial value per ton. As the proportion of top to root is subject to variation in different seasons and on different soils, it will be necessary, in order to arrive at the manurial value per acre, to weigh the tops from a small plot of known area, and then, by simple calculation, the desired value may be worked out.

Before concluding this account, the writers would like to take this opportunity of thanking all those farmers, too numerous to mention here individually, who so willingly supplied samples of sugar beet tops for the purposes of this investigation.

Appendix. *Nitrogen and Mineral Content of Samples of Sugar Beet Tops.*  
(Results expressed as percentages of dry matter.)

(a) From light and gravel soils.

N %	Ash %	SiO <sub>2</sub> %	P <sub>2</sub> O <sub>5</sub> %	CaO %	K <sub>2</sub> O %	Na <sub>2</sub> O %
2.08	17.30	2.63	0.643	1.70	2.83	3.59
1.86	17.34	3.04	0.526	1.84	2.79	2.90
1.91	16.38	0.24	0.580	1.95	1.90	3.64
1.78	22.20	5.23	0.726	2.09	5.96	1.25
1.78	19.40	4.88	0.967	1.77	5.29	3.33
2.00	18.89	3.15	0.589	1.83	4.99	3.50
1.94	16.22	2.40	0.572	1.79	3.18	2.46
2.51	23.01	5.69	0.689	3.01	3.28	3.25
1.51	25.73	15.20	0.548	1.10	3.03	1.21
2.59	19.86	9.73	0.967	1.49	2.30	1.85

(b) From medium soils.

1.72	15.31	3.49	0.470	1.92	3.56	2.96
1.77	18.34	1.16	0.399	3.33	4.68	3.70
2.06	21.58	7.72	0.653	1.20	3.23	3.52
2.26	18.78	2.99	0.716	1.45	2.81	4.78
1.90	14.07	0.71	0.492	2.72	2.42	2.52
1.75	20.03	8.08	0.563	3.19	2.56	0.85
1.48	26.00	14.82	0.686	2.35	2.25	1.14

(c) From heavy soils.

1.56	15.37	1.64	0.538	1.45	4.77	1.18
2.67	19.60	1.57	0.572	5.85	2.29	2.94

(d) From fen soils.

2.78	13.68	0.10	0.352	2.07	1.70	2.88
2.98	17.66	0.51	0.488	1.91	3.61	3.77
1.56	14.22	0.23	0.581	1.15	4.31	1.79
1.97	18.16	0.31	0.384	1.74	4.07	3.91
2.37	15.29	0.42	0.612	0.95	4.14	3.05
2.63	18.22	1.55	0.535	3.20	2.19	3.49

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# SOIL TEMPERATURES UNDER COTTON IN EGYPT.

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(With Five Text-figures.)

## INTRODUCTION.

IN 1924, continuous records of soil temperatures in fallow land were being made at Giza, Egypt. It was decided to supplement these observations by soil temperature readings under cotton. The records are of interest as they indicate the temperature conditions during germination and to which the roots are subjected during the periods of vegetative growth, flowering, boll development and maturation.

## METHOD OF RECORDING SOIL TEMPERATURES.

The instruments used for recording the soil temperatures were recording thermometers supplied by Messrs Negretti and Zambra. A pit 2 metres deep and 6 metres long was lined with cement. A concrete shelf was made along the south side of the pit 150 cm. above the floor.

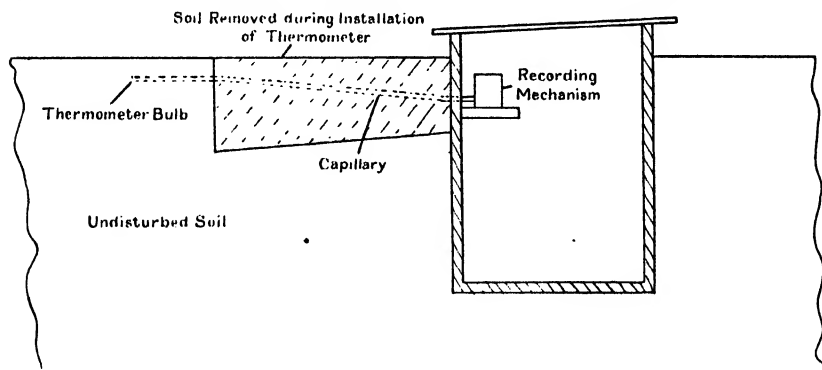


Fig. 1. Section through Soil Thermometer House.

The pit was covered with a tiled roof raised very slightly above the soil surface. The recording portions of the series of thermographs were placed on the concrete shelf and holes drilled in the cement wall through which the capillaries were passed into the soil outside. The earth between the pit wall and the soil in which the temperatures were to be recorded was dug out and an iron bar, the same diameter as the bulb of the thermometer, was driven horizontally into the undisturbed soil face at various depths from the surface. The thermometer bulbs were

inserted into the holes thus made in the undisturbed soil. The earth which had been removed was replaced over the capillaries. A diagrammatic section of the thermograph house and thermographs is given in Fig. 1. The instruments were checked for time daily and the charts changed at 8 a.m. on Monday of each week.

#### CULTIVATION OPERATIONS.

The cotton was sown on the flat at distances of 75 cm. between rows and 30 cm. between holes, the sowing depth being approximately 5 cm. The plants were thinned, two plants being left in each hole. The cotton was sown on March 16 and irrigated. The cultivations and irrigations were those normal to the crop. Irrigations were given on the following dates: May 1, May 30, June 19, July 3 and 24, August 7 and September 4. Flowering commenced during the week ending June 9. The crop was picked on September 15. The temperature records were taken over a period of 27 weeks from sowing to picking.

#### THE TEMPERATURE RECORDS.

The temperatures were recorded at depths of 10 and 20 cm. under the crop. For comparison, the temperatures recorded in the fallow soil

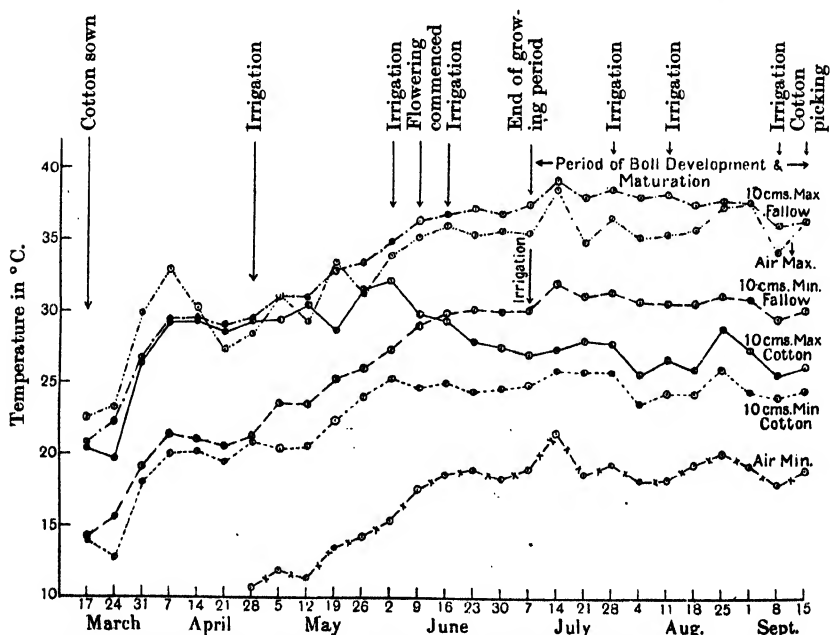


Fig. 2. Temperatures in Air and in Land under Cotton and Fallow. Depth—10 cm.

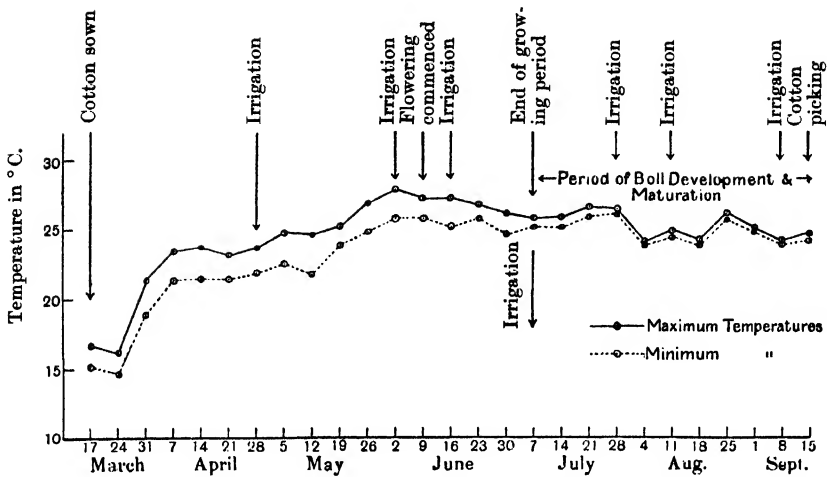


Fig. 3. Soil Temperatures under Cotton. Depth—20 cm.

Table I. *Soil Temperatures under Cotton, in Fallow Land, and Air.*

Week ending	Cotton				Fallow		Air	
	10 cm.		20 cm.		10 cm.			
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Mar. 17	20.4	14.0	16.7	15.2	20.8	14.3	22.5	6.5
" 24	19.7	12.8	16.2	14.7	22.2	15.7	23.3	8.6
" 31	26.5	18.1	21.4	18.9	26.7	19.2	29.9	10.8
Apr. 7	29.2	20.1	23.5	21.4	29.4	21.4	32.9	12.0
" 14	29.3	20.2	23.7	21.5	29.5	21.1	30.2	9.7
" 21	28.5	19.6	23.2	21.5	29.0	20.6	27.3	9.4
" 28	29.3	20.9	23.7	21.9	29.5	21.3	28.4	10.8
May 5	29.4	20.4	24.8	22.6	31.0	23.6	31.0	12.0
" 12	30.4	20.6	24.6	21.8	30.9	23.5	29.3	11.5
" 19	28.7	22.4	25.2	23.9	32.8	25.3	33.4	13.6
" 26	31.5	24.1	26.9	24.8	33.4	26.0	31.2	14.3
June 2	32.1	25.3	27.9	25.8	34.9	27.3	33.9	15.5
" 9	29.8	24.7	27.3	25.8	36.3	29.0	35.2	17.6
" 16	29.4	25.0	27.3	25.2	36.8	29.8	36.0	18.7
" 23	27.9	24.4	26.8	25.8	37.2	30.1	35.3	19.0
" 30	27.5	24.6	26.1	24.7	36.9	30.0	35.7	18.4
July 7	27.0	24.8	25.8	25.2	37.5	31.1	35.5	19.0
" 14	27.4	25.9	25.9	25.1	39.2	31.9	38.6	21.6
" 21	28.0	25.8	26.6	25.9	38.0	31.1	34.9	18.7
" 28	27.8	25.8	26.4	26.1	38.6	31.4	36.6	19.4
Aug. 4	25.6	23.6	24.1	23.8	38.0	30.7	35.1	18.2
" 11	26.7	24.3	24.9	24.5	38.2	30.6	35.4	18.4
" 18	26.0	24.3	24.2	23.8	37.5	30.5	35.8	19.5
" 25	28.9	26.1	26.2	25.7	37.8	31.1	37.4	20.3
Sept. 1	26.4	24.5	25.1	24.9	37.7	30.9	37.7	19.4
" 8	25.7	24.1	24.2	23.9	36.1	29.6	34.2	18.0
" 15	26.4	24.6	24.7	24.2	36.5	30.2	36.5	19.0



at 10 cm. depth, and also the air (screen) temperatures are given. The records are expressed as weekly means. All temperatures are in degrees centigrade. The records are shown in Table I and Figs. 2 and 3.

#### TEMPERATURE OF SEED BED.

The maximum temperature of the seed bed on March 16 was 22.5° C. and the minimum temperature was 11.8° C. The mean maximum temperature of the seed bed during the period of germination was 28.9° C. and the mean minimum temperature 12.2° C., giving a mean daily range of 16.7° C. It will be seen that the seed is subjected to a considerable daily range of temperature during the germination period. The mean daily range in the air (screen) temperatures during the same period was 14.7° C.

#### *The Maximum Temperatures at 10 cm. Depth.*

For convenience, the temperatures will be considered with reference to the distinctive periods in the plant's history—the period of vegetative growth, the flowering period, and the boll development and maturation periods.

*The Period of Vegetative Growth.* The period of vegetative growth extended from March 16 until approximately July 7. At the beginning of this period, from March 16 to May 5, there is at first a rapid rise in the maximum soil temperatures at a depth of 10 cm. and then they become almost stationary. During this period, the plant is exerting little effect on the soil temperatures as the curve for the corresponding depth in the fallow soil is practically identical with that under cotton. From that point, however, the curves for the cropped and fallow soil diverge, indicating that the shading effect of the plant is now coming into operation, though it does not become great until after June 2. From June 2 until July 7 the maximum temperatures gradually decline until they reach their minimum for this period of the crop growth. This point indicates the maximum shade effect of the crop and, hence, the termination of the period of vegetative growth. The period of vegetative growth may, therefore, be divided into two portions with reference to soil temperatures (a) a period from sowing until May 5, when the plants are mainly increasing in height and exerting little effect on soil temperatures, and (b) a period from May 5 until July 7 when branches are developing and during which the shading effects increase to a maximum.

*The Flowering Period.* The flowering period corresponds to the second portion of the period of vegetative growth during which branch development is taking place. The first flower was noticed during the week ending June 9. At the beginning of the flowering period the maximum soil temperatures at 10 cm. have reached the highest point which they attain during the time that the land is occupied by cotton. The shading effect of the plant increases during the flowering period so that the maximum soil temperatures at 10 cm. are characterised by a continuous decline to an almost constant value.

*The Boll Development and Maturation Periods.* At the beginning of the boll development period the maximum soil temperatures have fallen considerably to an almost constant level which is maintained throughout the maturation period. The variations in the maximum temperatures which do occur are of small magnitude and are directly attributable to irrigation. The temperatures after irrigation rapidly return to the constant value which is approximately 26° C. The boll development and maturation periods are, therefore, characterised by a constant maximum soil temperature at a depth of 10 cm.

*The Minimum Soil Temperatures at 10 cm. Depth.*

The minimum temperatures are affected by the plant in a different manner to the maximum temperatures. Until June 2 the plant exerts little effect on the minimum soil temperatures, the curve for the soil under cotton being almost identical with the curve for the fallow soil except where influenced by irrigation. After June 2, the curve for the maximum soil temperature at 10 cm. begins to fall and at the same point the curve for the minimum soil temperatures suddenly diverges from the corresponding curve for the fallow soil. The re-adjustment is, however, rapid, and the minimum temperature curve under cotton again becomes parallel to the fallow soil curve though at a lower temperature. As the curve for the minimum temperatures in the soil under cotton is, in general, parallel to the curve for the minimum temperatures in fallow soil, it appears that the plant exerts little effect on radiation during the night which is the main factor determining the minimum temperature.

*The Period of Vegetative Growth.* During the initial stages of this period, the minimum soil temperatures at 10 cm. rise rapidly in a similar manner to the minimum temperatures in the fallow soil. The irrigation of May 1 exerts a small depressing effect but the soil temperatures rapidly recover until the minimum reaches its highest point for the season on June 2. From June 2 to the end of the period of

vegetative growth the minimum temperatures are almost constant, the curve after June 16 running parallel to that for the fallow soil. The vegetative period is, therefore, characterised by an initial rapid rise in the minimum temperatures followed by a period of constant minimum. There is no decline in the minimum temperatures corresponding to the decline in the maximum temperatures after June 2.

*The Flowering Period.* At the beginning of the flowering period the minimum soil temperatures at 10 cm. depth have reached their highest point and remain almost constant throughout the period at about 25° C. The flowering period is, therefore, characterised by a constant minimum soil temperature.

*The Boll Development and Maturation Periods.* During these periods, the minimum soil temperatures at 10 cm. continue to remain constant at approximately 25° C. except when influenced by irrigation. The maximum irrigation effect is small, being a depression of 2° C. from which the soil temperatures rapidly recover. The boll development and maturation periods are, therefore, characterised by constant minimum soil temperatures at 10 cm.

*The Amplitude of the Daily Temperature Wave at 10 cm.*

The weekly means of the daily range of temperature are shown in Fig. 4, the air range and fallow soil range being also given for comparison. It will be seen that the soil temperature range at 10 cm. is divisible into three distinct sections: (a) March 16 to May 12, (b) May 12 to July 14 and (c) July 14 to September 15. The temperature range increases during the first of these periods in a similar manner to that of the fallow soil. The maximum daily temperature range during the growth of the crop is attained during this period. The second period is characterised by a gradual reduction in temperature range which coincides with the development of branches. The fallow soil range has now reached a constant value which it maintains throughout the period under discussion. During the third period, which corresponds to that of boll development and ripening, the temperature range is small and is almost constant.

There is a close resemblance between the type of range in the fallow soil and that in the air. Both show the initial increase and thereafter remain constant. The range of temperature in the air is, however, considerably greater than that in the fallow soil at 10 cm. depth.

During germination and the period characterised mainly by increase in height of the plant, the soil temperature range at 10 cm. increases

from approximately 6° C. at the beginning of the period to 10° C. at the end of the period. During the production of branches and flowers, the range gradually decreases from 10° C. to 2° C. at the end of the period. Throughout the period of boll development and maturation the range remains approximately constant at 2° C.

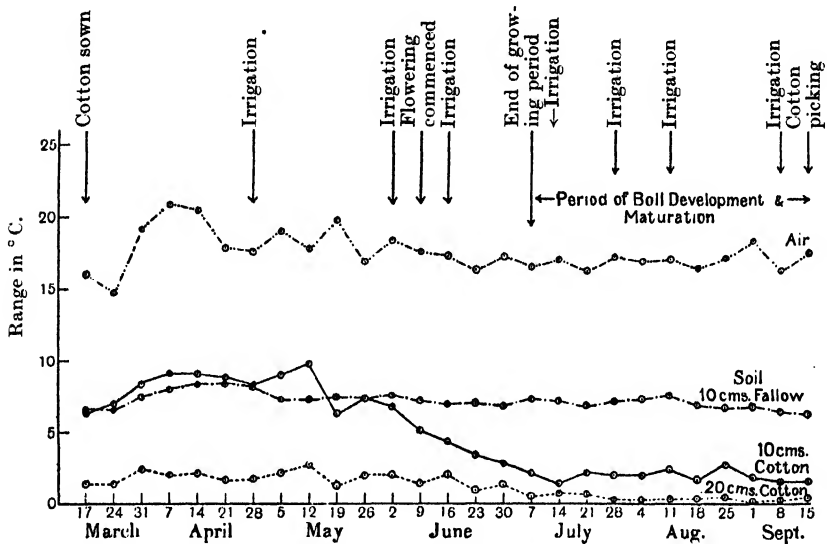


Fig. 4. Daily Range of Temperature (Weekly Means) in Air and Soil.

#### *The Maximum Temperatures at 20 cm. Depth.*

The temperatures at a depth of 20 cm. are of considerable importance as this is the depth of maximum lateral root development of the cotton plant. The curves for the soil temperatures at 20 cm. exhibit similar characteristics to those at 10 cm. though not to the same degree.

The curve for the maximum soil temperatures at 20 cm. shows a considerable rise during the period of seed germination. The temperatures then continue to rise gradually to their seasonal maximum which occurs just before the period of maximum branch formation. From this point to the end of the period of vegetative growth, a small decline in the maximum takes place and it then remains constant, except where influenced by irrigation, until the end of the maturation period. The maximum temperature recorded was 28° C., the constant temperature during flowering, boll development and maturation being approximately 26° C.

*The Minimum Temperatures at 20 cm. Depth.*

The minimum temperatures at 20 cm. rise rapidly during the germination stage of the plant. The rise then becomes more gradual during the period characterised by the increase in height of the plant. The minimum temperatures reach their highest point for the season at the commencement of the flowering period. From the beginning of the flowering period to the end of the maturation period they remain almost constant. This constant temperature is about 26° C.

*Amplitude of Temperature Wave at 20 cm. Depth.*

The daily range of temperature at a depth of 20 cm. is small throughout the period that cotton is on the land, never exceeding 2.5° C. The range is approximately constant at 2° C. until June 2, the commencement of the flowering period. After June 2, the range gradually decreases with the declining maximum until the end of the period of vegetative growth. Throughout the periods of boll development and maturation the daily temperature range is almost constant at 0.5° C.

*Soil Temperatures at Depths below 20 cm.*

Below a depth of 20 cm. records are only available for the temperatures at 50 cm. At this depth a daily range of 0.4° C. is apparent at the beginning of the period. This daily variation is eliminated by the end of April. The temperature rises to 25° C. on May 2 and remains practically constant until the end of the maturation period. From July 7 until the end of the maturation period, the temperature of the soil between 10 cm. and 50 cm. remains almost constant at between 25° C. and 27° C., there being little variation in temperature with increasing depth and no point in this depth having a greater daily variation than 2° C.

**THE EFFECT OF IRRIGATION ON SOIL TEMPERATURES.**

The effect of irrigation on soil temperatures in fallow land has been dealt with previously<sup>(1)</sup>. It has been shown that the effect of irrigation on soil temperatures is due to the displacement downwards of the water already in the soil by the irrigation water. The actual effect of an irrigation on soil temperatures, therefore, depends on the temperature conditions in the soil at the time of irrigation. As an example of the effect of irrigation on soil temperatures that of the week ending July 16 may be taken. The temperature of the irrigation water was 27.9° C. The result of the irrigation on the temperatures at 10 cm. and 20 cm. is

shown in Fig. 5. The irrigation took place at 9 a.m., the minimum temperature at 10 cm. having been reached at 8 a.m. At 10 cm. the effect of the irrigation has been to stop the normal rise in temperature, but no fall in temperature occurred. The temperature started to rise again about 11 a.m. and continued to rise until the time of normal maximum temperature at 2.30 p.m. The main effect of irrigation has been to reduce the amplitude of the temperature wave at 10 cm. This is shown

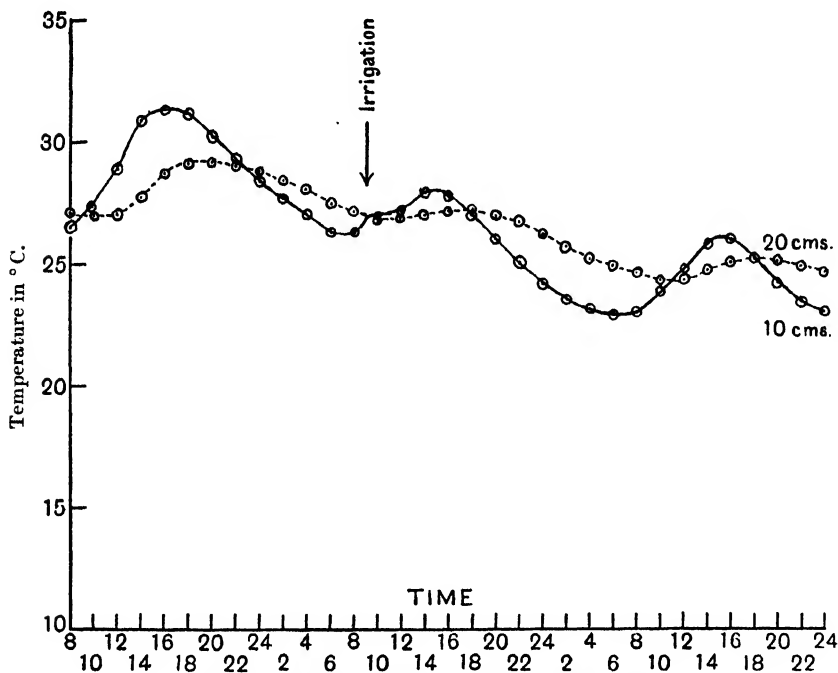


Fig. 5. Effect of Irrigation on Soil Temperatures.

in Fig. 5 in which the temperatures for the day before irrigation, the day of irrigation, and the day following irrigation are given. At 20 cm. the irrigation has produced an artificially early minimum at 10 a.m. instead of 11 a.m. the normal time of minimum at this depth. The irrigation has again produced no cooling effect on the soil temperatures. It will also be seen that the irrigation has produced a damping effect on the temperature wave, no fall or rise in temperature is, however, noticeable. The main effect of irrigation on the soil temperatures under cotton is to reduce the amplitude of the daily temperature wave at depths of 10 cm. and 20 cm.

## DISCUSSION OF ROOT TEMPERATURE CONDITIONS.

The soil temperatures represent the temperature conditions under which the roots of the cotton plant are situated. The greatest variations in the soil temperatures, both as regards increase in temperature and the amplitude of the temperature wave, occur during the germination of the seed and the initial stages of growth. The plant normally pushes its roots rapidly through the layer of soil liable to large variations in temperature and develops its lateral system of roots at depths of 20 cm. and below. The soil zone in which lateral root development takes place is characterised by three sets of temperature conditions during the period of root development and activity. The first set of conditions is a gradual rise in the maximum and minimum temperatures until the commencement of the flowering period. The amplitude of the temperature wave during this period is large. The second set of root temperature conditions are those associated with the branch development and flowering periods of the plant. During these periods the maximum soil temperatures decline, the minimum temperatures remaining constant. The main characteristic of these periods is the reduction in the amplitude of the daily temperature wave following the gradual reduction in the maximum temperature. The third set of root temperature conditions are those associated with the boll development and maturation period. During this period the maximum temperature remains constant, the minimum temperature remains constant and, consequently, the roots are subjected to a very small constant daily range of temperature. It will be seen that during the important periods of branching, flowering, boll development and maturation the plant itself controls to a large extent the temperature factor of its root environment.

The constant soil temperature at 10 cm. during the boll development and maturation periods is between 25° C. and 27° C. The constant temperature at 20 cm. during the same periods is 26° C. and at 50 cm. it is also about 26° C. The whole of the lateral root system and a considerable portion of the tap-root of the cotton plant is, therefore, at a constant temperature of 26° C. during the boll development and maturation periods. That the whole of the root system of the plant is at the same temperature during the boll development and maturation periods may be of special significance in connection with the physiological processes during seed production.

The temperature conditions to which the root system of the cotton plant is subjected are in striking contrast to those of the overground

system as indicated by the air temperatures. The overground portion of the plant is subjected to a high rise of temperature during the initial stages of growth and to almost constant maximum and minimum temperatures during the boll development and maturation periods. The most important difference in the temperature conditions is brought out when the amplitude of the air temperature wave is considered. As has been shown, the temperature wave to which the roots are subjected is practically eliminated during the period of vegetative growth. The overground system, however, experiences a considerable daily range of temperature. The air temperature range, as indicated by the screen thermometer, is practically constant at  $17^{\circ}$  C. throughout the boll development and maturation periods.

#### GENERAL APPLICATION OF RESULTS.

The results of the investigation in Egypt may be applied directly to other cotton growing countries. Although the actual maximum and minimum soil temperatures cannot be directly inferred from the maximum and minimum air (screen) temperatures, it appears from the study of the fallow soil records that the range of soil temperature bears a direct relationship to the range of air temperature during periods in which rainfall is small. In cotton producing countries the rainfall is small during the boll development and maturation periods. A constant range of air temperature during the boll development and maturation periods will, therefore, indicate a constant range of soil temperature.

Williams<sup>(2)</sup> has shown, from a consideration of the air temperatures, that the cotton growing countries fall into three groups:

- (1) Countries in which cotton is grown at the warm time of the year between two periods of low temperature.
- (2) Countries in which cotton is grown at a cool time of the year between two periods of high temperature.
- (3) Countries in which the temperature is more or less constant throughout the year.

Table II shows the ranges of air temperature during the various periods of the history of the cotton plant in countries of the above types. The figures have been obtained by measurement on the diagram in Williams' paper.

It will be seen that the range of air temperature at sowing time varies considerably in the three types of cotton growing countries. During the periods of vegetative growth and flowering, the ranges of air temperature to which the cotton plants are subjected vary from



Table II. *Monthly Means of Daily Range of Air Temperature in Cotton Producing Countries (degrees centigrade).*

	Country	Sowing	Vegetative growth and flowering			Boll development and maturation		
<i>Type 1</i>	Egypt	16.0	18.0	18.0	18.0	17.0	16.0	15.0
	Texas	13.0	14.0	14.0	15.0	13.0	13.0	14.0
	Turkestan	12.0	15.0	14.0	16.0	19.0	19.0	18.0
	Mesopotamia	15.0	16.0	16.0	19.0	19.0	20.0	21.0
<i>Type 2</i>	Sudan	16.0	15.0	13.0	12.0	13.0	14.0	15.0
	Madras	10.0	9.0	7.0	9.0	11.0	9.0	10.0
<i>Type 3</i>	Nigeria	5.0	6.0	6.0	7.0	8.0	8.0	7.0

country to country but tend to approach a constant value at the end of the period. This constant air range is maintained with small variation throughout the boll development and maturation periods. Mesopotamia is no exception to the above though Williams states that it differs considerably from the remaining cotton growing countries in other respects.

It can be directly inferred from the air temperatures that during the periods of boll development and maturation the soil temperatures under cotton are subject to an almost constant daily range. Further, it may be inferred that this soil temperature range in the zone of maximum root development is small as in the cotton growing countries the air range is either similar to or smaller than that of Egypt. The position of this constant temperature range on the thermometric scale will vary and may have some bearing on the type of cotton grown. It seems probable, therefore, that the characteristics of the soil temperatures during the boll development and maturation periods of cotton are common to all cotton producing countries.

#### SUMMARY.

The results of the investigation may be summarised as follows:

(1) In Egypt, during the periods of germination and increase in height of the cotton plant, the soil temperatures are rising and the amplitude of the temperature wave is at its maximum.

(2) In Egypt, the maximum soil temperatures decline while the minimum soil temperatures remain constant, thus resulting in a gradual decrease in the amplitude of the daily temperature wave during the branching and flowering periods.

(3). In Egypt, throughout the soil zone occupied by the roots of the plant, the temperature is the same, the amplitude of the daily temperature wave small, and the temperature is constant during the boll development and maturation periods.

(4) The main effect of irrigation on soil temperatures is to reduce the amplitude of the daily temperature wave, no sudden change of temperature in the root zone taking place.

(5) From a consideration of the range of air temperature in other cotton producing countries, it seems probable that the characteristics of the soil temperatures during the boll development and maturation periods are the same for all countries.

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# THE VARIATIONS IN MILK YIELDS CAUSED BY SEASON OF THE YEAR, SERVICE, AGE, AND DRY PERIOD AND THEIR ELIMINATION.

## PART II. SERVICE.

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(With Eight Text-figures.)

### SECTION A. THE EFFECT OF THE LENGTH OF THE S.P. ON THE TOTAL LACTATION YIELD.

*All Cows.* It is proposed, in dealing with this question, to follow the same lines as in the previous Part—namely, to describe the effect as found from all the Norfolk records (comparing the results, where possible, with those found in the case of Penrith) and then to treat the various breeds, and high and low yielders separately, in order to bring out any peculiarities that may exist as regards those groups.

It has already been seen that, as would be expected, the length of the S.P. very largely influences the length of the lactation, and it would seem probable on the face of it, that as pregnancy progresses the milk flow declines; this latter is dealt with in Section B of this Part, but we are here concerned with the total effect of both of these on the yield of milk in the lactation.

The work described in Part I showed that the effect of the length of the S.P. on the length of the lactation in the case of 1st calvers, differs considerably from the effect on older cows—that the former, on the average, milk for a fortnight longer than the latter; further, it will be shown later that heifers have a distinctive type of lactation curve—a long flat one. Under these circumstances it would appear probable that, though the effect here dealt with may be of the same form, it will be different in extent for 1st calvers and others. This expectation was fulfilled in the correlation tables, between the length of the S.P. and the lactation yield, that were drawn up for these two groups separately. Both tables showed a fairly well-marked positive relationship between long S.P.'s and high yield, and the mean yields for the different S.P. intervals rose quite regularly. Table XIII gives the usual statistical constants obtained from the two correlation tables.

Table XIII. *Correlation between Length of S.P. and Total Lactation Yield.*

Constant	1st calvers	Older cows
Mean yield	6173 $\pm$ 41 lb.	7165 $\pm$ 26 lb.
Standard deviation of yield	1785 $\pm$ 29 lb.	2112 $\pm$ 18 lb.
Mean S.P.	89.9 $\pm$ 1.3 days	81.8 $\pm$ 0.6 days
Standard deviation of S.P.	58.5 $\pm$ 0.9 days	53.1 $\pm$ 0.4 days
$r$	+0.444 $\pm$ 0.018	+0.407 $\pm$ 0.010
$\eta$ (yield, S.P.)	0.494 $\pm$ 0.017	0.427 $\pm$ 0.010
$\eta^2 - r^2$	0.0469 $\pm$ 0.0098	0.0175 $\pm$ 0.0033
Regression equation (yield on S.P.)	$y = 4955 + 13.55 \cdot x$	$y = 5841 + 16.19 \cdot x$
Equation of fitted curve	$y = 10,000 - 5395 \cdot e^{-0.004007 \cdot x}$	$y = 11,000 - 5821 \cdot e^{-0.005410 \cdot x}$
	$y$ = yield in lb.	$x$ = S.P. in days.

In each case there is a positive value of  $r$  of about 0.4, which is many times its probable error; the value is slightly higher for 1st calvers, but the difference is only  $0.037 \pm 0.021$  and so should not be regarded as trustworthy; in the same way the correlation ratio is slightly higher for 1st calvers, but, again, the difference is insignificant, being  $0.067 \pm 0.020$ .

The values of  $\eta^2 - r^2$ , which are regarded as tests of the linearity of the regressions, are rather low, but their probable errors show that they both just differ significantly from zero—the ratio of  $\eta^2 - r^2$  to its probable error being 4.8 for 1st calvers, and 5.3 for the others.

This suggests that the relationship is not a linear one; neither would such be expected on commonsense grounds, for linearity would mean that an empty cow would eventually (on the average) settle down to a yield of 16 lb. a day (or  $13\frac{1}{2}$  lb. for heifers—cf. the regression equation), and that this yield would be maintained indefinitely. Whilst a number of instances might be cited of individuals that have kept up a good flow for very long periods (even for five years), it is not believed that the average cow under ordinary conditions would do so. Furthermore it was shown in Part I that as the length of S.P. increases the duration of the lactation after service decreases; this does not point to a linear relation in the present case.

As a matter of fact the mean yields for the successive S.P. intervals rose rapidly at first and then more slowly, apparently tending to a limit; this suggests that the relationship should be represented by a curve of the form

$$y = A - Be^{-Cx},$$

where  $y$  represents yield,  $x$  the S.P. and  $A$ ,  $B$  and  $C$  are constants,  $A$  being the limit towards which the means approach at the extreme end of the range of S.P.

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Trial gave the curves the equations of which are given in the last line of Table XIII and these are shown in Fig. 15—the broken line being the curve for 1st calvers, and the continuous one for the others, the actual means obtained being represented by small circles and crosses respectively.

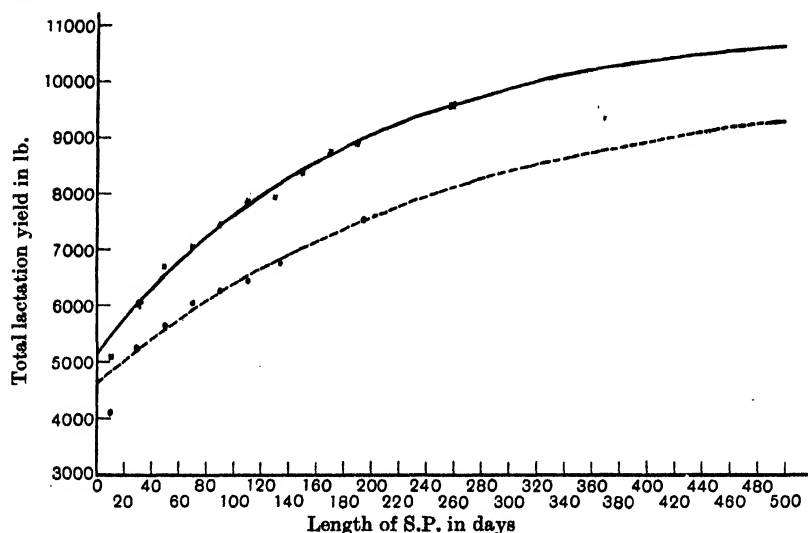


Fig. 15. Effect of the length of the S.P. on the total lactation yield (all cows).

1st Calvers - - - - - Means obtained ○  
Others - - - - - Means obtained x

Except for the S.P. interval 0–19 days the fit of these two curves is good; in each case the first point obtained is noticeably below the curve, but the numbers involved were small. The probable explanation would appear to be that these cows were rather below the average as milk producers, because high yielders can rarely be got in calf again so early in the lactation—even if desired. Hammond(4) has found in rabbits that where a litter of four or more is being suckled, it is impossible to get the doe pregnant again, but with only one or two being suckled this can be accomplished—*i.e.* that a large output of milk inhibits or delays ovulation. The fact might also be cited that in the human subject menstruation is often in abeyance during lactation—in fact it is disputed whether this, or its occurrence, is the normal(6). In view of these and other facts, it would seem justifiable to conclude that these few cows were rather below the average and consequently that the curves represent the true function; this necessitates the assumption that good and bad cows were distributed evenly over the rest of the range of S.P.,

but there would appear to be no great difficulty in this, for we have already seen that there is no marked difference in the mean length of S.P. between high and low (total) or (maximum) yielders. Further evidence that these curves are not subject to error by reason of unequal distribution of different grades of cows will be adduced in Section B of this Part.

From these curves have been obtained corrections, by means of which allowance may be made for different lengths of S.P.—a percentage of the actual yield obtained being subtracted from those with long S.P.'s, and a percentage added to those with short ones, in order to estimate what would have been the yield, had the S.P. been of standard length; this standard length has been taken as 85 days, partly because that is about the average for all these lactations, but chiefly because a cow with a S.P. of 85 days will be due to calve again in exactly a year's time.

Table XIV. *Corrections for Length of S.P.*

S.P. in days	Yield from smoothed curve (lb.)		Percentage correction (to estimate yield with S.P. = 85 days)		
	1st calves	Others	1st calves	Others	Penrith
0-19	4806	5470	+ 28.2	+ 33.9	+ 26.0
20-39	5206	6037	+ 18.4	+ 21.3	+ 17.0
40-59	5575	6546	+ 10.6	+ 11.9	+ 9.7
60-79	5916	7000	+ 4.2	+ 4.6	+ 3.9
80-99	6230	7410	- 1.1	- 1.1	- 1.0
100-119	6521	7781	- 5.5	- 5.9	- 5.0
120-139	6789	8111	- 9.2	- 9.7	- 8.5
140-159	7036	8408	- 12.4	- 12.9	- 11.4
160-179	7264	8673	- 15.2	- 15.5	- 14.0
180-199	7475	8912	- 17.6	- 17.8	- 16.2
200-219	7669	9126	- 19.7	- 19.7	- 18.1
220-239	7849	9319	- 21.5	- 21.4	- 19.7
240-259	8014	9491	- 23.1	- 22.8	- 21.2
260-279	8167	9646	- 24.5	- 24.1	- 22.5
280-299	8308	9784	- 25.8	- 25.1	- 23.7
300-319	8439	9909	- 27.0	- 26.1	- 24.7
320-339	8559	10021	- 28.0	- 26.9	- 25.6
340-359	8670	10122	- 28.9	- 27.6	- 26.5
360-379	8772	10212	- 29.8	- 28.3	- 27.2
380-399	8867	10293	- 30.5	- 28.8	- 27.9
400-419	8954	10365	- 31.2	- 29.3	- 28.5
420-439	9035	10430	- 31.8	- 29.8	- 29.0
440-459	9109	10489	- 32.4	- 30.2	- 29.5
460-479	9178	10541	- 32.9	- 30.5	- 29.9
480-499	9241	10588	- 33.3	- 30.8	- 30.3
85	6162	7325	—	—	—

In the first column of Table XIV is shown for 1st calves the value given by the smooth curve, for the S.P. interval on the left, and the second the value for older cows similarly; next follows the corrections for 1st calves, others, and those found from the Penrith data. The

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corrections were found by dividing the value given by the curve for the standard S.P. (at the foot of columns 1 and 2), by the value for the interval—e.g. for the interval 0–19 days (1st calvers) we have  $\frac{4162}{4806} = 1.282$ , i.e. if 28.2 per cent. be added on to 4806 we arrive at the figure 6162. The actual figures for Penrith have been given before (5) but the corrections were worked out for a standard S.P. of 100 days; the present standard seems preferable, and so for purposes of comparison they have been calculated afresh on that basis; the curve for Penrith gave a figure of 5441 lb., corresponding to a S.P. of 85 days.

The variation of S.P. is of course a continuous one and greater accuracy may be obtained by finding the exact correction for each S.P. by interpolation; only a small error, however, is made by using the actual figures for the whole interval, except for very short S.P.'s when those figures are falling rapidly; for a S.P. of 19 days (1st calvers), by ordinary linear interpolation we arrive at the figure + 23.5 per cent., which would be more accurate than the + 28.2 per cent. given for the interval—but this is, of course, an extreme case. In doing this it must be realised that the intervals are 0–19 days, 20–39 days, etc., and consequently the figures given, which correspond to the mid points of the intervals, are actually for S.P.'s of 9.5 days, 29.5 days and so on.

The difference in the two sets of corrections for 1st calvers and others is not very large, but just large enough to warrant the use of different sets; it will be noticed that the correction needed is greater for older cows for all S.P.'s up to 200 days, and then less. This accords exactly with what might have been predicted from a knowledge of the fact that older cows give a comparatively steep form of curve, and consequently a higher proportion of their yield in the early part of the lactation; under these circumstances a short S.P. will handicap a heifer less, whereas with a long one she will give a proportionately larger increase.

The Penrith corrections are numerically less over the whole range; this may be due to smaller numbers in that case giving a less perfect fit, or to the fact noticed in Part I that the conditions under which they are kept are notably worse. Superlative accuracy is hardly desirable as many things are liable to change a cow's yield by 1 or 2 per cent., and for all practical purposes the agreement is, at any rate, satisfactory.

In dealing with the Penrith records it was found that this effect varied slightly according to the month of calving; the fact emerged that the smooth logarithmic type of curve hardly described this function accurately for cows calving in August and September; with these cows there is a critical time for service about the end of November—if they

are served earlier they will, on the average, be dry before the following spring flush, if a week or two later, they will just last over it, and consequently give a considerably higher yield. To see if anything of this sort existed in the case of Norfolk, separate correlation tables, between the length of the S.P. and the lactation yield, were drawn up for all the cows calving in the several calendar months; unfortunately the numbers were insufficient to give any definite results, and so the tables are not given, but two small indications were noted and might be mentioned; where a high yield in the lactation is the determining aim it appears that:

(1) Cows that would normally be served in November should be left till mid-December—*i.e.* as was found in the Penrith data; in Norfolk this was not, however, so clear cut, presumably because of the less pronounced rise in yield associated with the spring flush of grass in that district.

(2) Cows that would normally be served in April might, with advantage, be left till the end of May or early June, but little further gain appears to be made by leaving them longer than that—unless they be kept empty until September.

*Breeds.* Correlation tables between the length of the S.P. and the lactation yield were drawn up for Mongrels, Red Polls, Lincoln Reds and Friesians separately, but limitations of space forbid their reproduction; the paucity of numbers did not allow of the division of 1st calvers from others. The statistical constants derived from these tables are given in Table XV.

Table XV. *Correlation between Length of S.P. and Total Lactation Yield.*

Constant	Mongrels	Red Polls	Lincoln Reds	Friesians
Mean yield	7071 $\pm$ 34 lb.	6639 $\pm$ 56 lb.	6629 $\pm$ 55 lb.	7899 $\pm$ 86 lb.
Standard deviation of yield	2032 $\pm$ 24 lb.	2116 $\pm$ 40 lb.	1792 $\pm$ 39 lb.	2490 $\pm$ 61 lb.
Mean S.P.	79.4 $\pm$ 0.8 days	82.9 $\pm$ 1.5 days	87.7 $\pm$ 1.8 days	93.3 $\pm$ 2.1 days
Standard deviation of S.P.	48.3 $\pm$ 0.6 days	56.9 $\pm$ 1.1 days	59.3 $\pm$ 1.3 days	61.4 $\pm$ 1.4 days
$r$	+0.419 $\pm$ 0.014	+0.350 $\pm$ 0.023	+0.425 $\pm$ 0.025	+0.410 $\pm$ 0.029
$\eta$ (yield, S.P.)	0.435 $\pm$ 0.013	0.443 $\pm$ 0.021	0.469 $\pm$ 0.024	0.470 $\pm$ 0.027
$\eta^2 - r^2$	0.0136 $\pm$ 0.0039	0.0738 $\pm$ 0.0145	0.0395 $\pm$ 0.0123	0.0528 $\pm$ 0.0158
Regression equation (yield on S.P.)	$y = 5671 + 17.63.x$	$y = 5560 + 13.02.x$	$y = 5503 + 12.84.x$	$y = 6342 + 16.68.x$
Equation of fitted curve	$y = 10500 - 5105.e^{-0.005536.x}$	$y = 9500 - 4525.e^{-0.005734.x}$	—	—
	$y = \text{yield in lb.}$		$x = \text{S.P. in days.}$	

The value of  $r$  is remarkably constant for three of the breeds, but it is rather low for Red Polls; however, the difference in the value from



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Red Polls to Mongrels is only  $0.069 \pm 0.027$  and so should not be regarded as significant; similarly the values of  $\eta$  do not show any marked variation from breed to breed;  $\eta^2 - r^2$  is small and only differs significantly from zero for Red Polls, in which case it is equal to 5.1 times its probable error. Nevertheless, despite the low value of  $\eta^2 - r^2$ , the function did not appear to be linear for Mongrels and a logarithmic curve was found to give a good fit to the data.

With Lincoln Reds and Friesians there appeared to be no fault to find with the fit of the actual regression lines, but it is difficult to believe that there is any real difference in this respect between these and the other two breeds; it will be noticed that in the two cases where the largest numbers were involved (1661 and 642) the regressions were logarithmic in form, whilst it was only where there was a small number of observations (476 and 383), that there was any apparent linearity.

Separate corrections for each breed were calculated and are given in an abbreviated form in Table XVI.

Table XVI. *Corrections for Length of S.P.—calculated for Breeds Separately.*

Length of S.P. (mid-value of interval) Days	Percentage corrections (standard S.P. = 85 days)			
	Mongrels (logarithmic)	Red Polls (logarithmic)	Lincoln Reds (linear)	Friesians (linear)
9.5	+29.3	+28.8	+17.2	+19.4
29.5	+18.6	+18.3	+12.1	+13.6
49.5	+10.5	+10.3	+7.4	+8.3
69.5	+4.1	+4.0	+3.5	+3.5
89.5	-1.0	-1.0	-0.9	-0.9
109.5	-5.2	-5.1	-4.6	-5.0
149.5	-11.6	-11.3	-11.2	-12.2
209.5	-17.8	-17.4	-19.5	-21.1
309.5	-23.7	-23.0	-30.4	-32.5
409.5	-26.7	-25.9	-38.7	-41.1
489.5	-28.0	-27.2	-44.1	-46.5

Where a curve has been used the agreement is extremely good; in no case is there a difference of 1 per cent. in the figures for Mongrels and Red Polls, and they agree well with the corrections already found for All Cows. For the other two breeds the corrections have been obtained from the regression lines, and are quite different; remembering, however, the constancy of the values of  $r$  and  $\eta$  in the various breeds, it would not be justifiable to claim that any definite breed peculiarity has been established; it is probable that little error would be introduced by the application of the corrections given in Table XIV to any breed of cow.

## HIGH AND LOW YIELDERS.

In order to test whether the relationship between the length of the S.P. and the lactation yield is the same with high yielding cows as with low yielders, correlation tables between these two variables were drawn up for the two groups that have been called high and low (total) yielders; it will be recalled that these two groups contained the best and the worst third respectively of the Mongrels, and that there were 552 lactations in each. The statistical constants derived from these correlations are given in Table XVII.

Table XVII. *Correlation between Length of S.P. and Total Lactation Yield.*

Constant	High (total) yielders	Low (total) yielders
Mean yield	8879 $\pm$ 55 lb.	5434 $\pm$ 35 lb.
Standard deviation of yield	1903 $\pm$ 39 lb.	1202 $\pm$ 24 lb.
Mean S.P.	82.1 $\pm$ 1.5 days	78.3 $\pm$ 1.3 days
Standard deviation of S.P.	51.9 $\pm$ 1.1 days	44.8 $\pm$ 0.9 days
$r$	+0.535 $\pm$ 0.020	+0.551 $\pm$ 0.020
$\eta$ (yield, S.P.)	0.559 $\pm$ 0.020	0.573 $\pm$ 0.019
$\eta^2 - r^2$	0.0263 $\pm$ 0.0093	0.0134 $\pm$ 0.0090
Regression equation (yield on S.P.)	$y = 7268 + 19.62.x$	$y = 4277 + 14.78.x$
Equation of fitted curve	$y = 13750 - 7014.e^{-.004812.x}$	$y = 7800 - 3860.e^{-.006880.x}$
	$y$ =yield in lb.	$x$ =S.P. in days.

There is a very close agreement in the values of  $r$ , and both are noticeably higher than for all the Mongrels; this obviously arises in the selection of the group on the basis of yield, the standard deviation of that variable being thereby greatly reduced. The correlation ratio is almost the same for each group and does not differ markedly from  $r - \eta^2 - r^2$  being insignificant in each case.

Despite this, however, logarithmic curves seem to fit the means obtained better than the regression lines, and two have been fitted and are shown in Fig. 16; these curves cut through the data well.

One rather peculiar point may be noticed about the results for high yielders—the mean for the interval 0–19 days lies a little above the curve; at first sight this seems to be at complete variance with the suggestion put forward (p. 504) that the usual low figure obtained for this interval is due to high yielders not often being successfully served so early in the lactation; it should be mentioned, therefore, that only three observations are included in this mean and seven in the corresponding one for low yielders—i.e. out of the same number in each group, over twice as many low yielders fall in this interval as high yielders. Such small numbers cannot give a reliable result, but it might be pointed

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out that if the actual numbers are taken into account, these two means support, rather than contradict, the above suggestion.

Corrections have been worked out from these curves for the different 20-day intervals of S.P. and are given in an abbreviated form in Table XVIII.

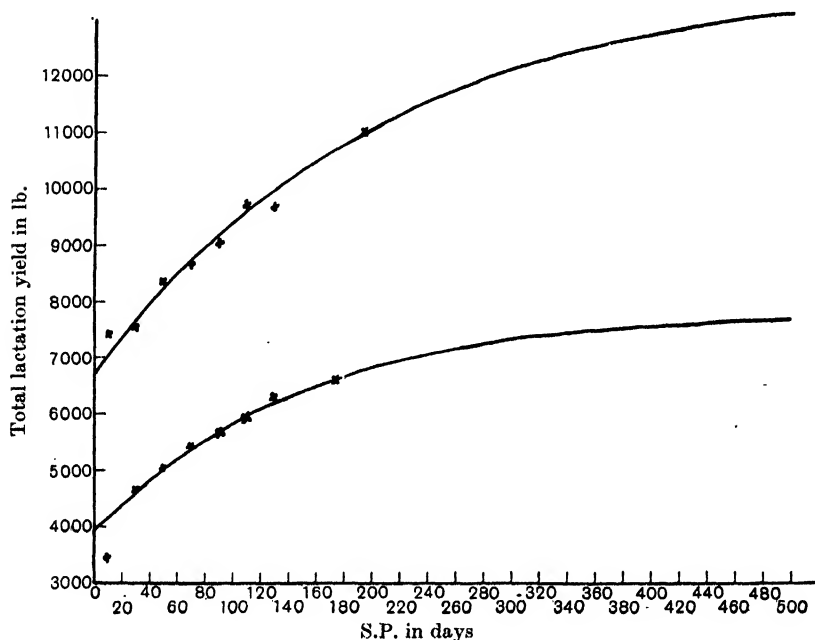


Fig. 16. Effect of the length of the S.P. on the total lactation yield.  
High and Low (total) yielders.

Table XVIII. *Corrections for Length of S.P.—calculated for High and Low Yielders separately.*

Length of S.P. (mid-value of interval)	Percentage corrections (Standard S.P. = 85 days)	
Days	High (total) yielders	Low (total) yielders
9.5	+28.9	+34.9
29.5	+18.5	+21.6
49.5	+10.7	+11.9
69.5	+4.1	+4.5
89.5	-1.1	-1.1
109.5	-5.6	-5.6
149.5	-12.0	-12.1
209.5	-18.8	-18.2
309.5	-25.3	-23.4
409.5	-28.8	-25.8
489.5	-30.5	-26.8

Except for the two ends of the S.P. distribution (under 40 days and over 200 days), there is little divergence between the two sets of corrections; the actual difference is almost exactly the same as that found between 1st calvers and others—the corrections for high yielders approximate to those for 1st calvers, and those for low yielders to the corrections for older cows. In the other case this difference was deemed to be due to the fact that the lactation curve for 1st calvers shows a low maximum but great persistence, whilst with older cows, a greater proportion of the yield is given in the early part of the lactation; if the mean curves for the present group be referred to (Part I) exactly the same thing appears—namely, that high (total) yielders give a persistent yield compared to the larger proportionate fall and earlier drying off of low (total) yielders. Under these circumstances it is only reasonable to conclude that this is the cause of the difference in the above two sets of corrections.

But persistence of yield is by no means confined to high yielders; high milk production depends on two things—high physiological capacity and great persistence of yield, and there does not seem to be a close connection between these two factors. A high yielding (total lactation yield) group of cows will show both capabilities, but among low yielders will be found not only individuals with a good physiological capacity that, however, fall off very rapidly in their flow, but others endowed with the persistency factor, but incapable of giving a high yield at any time. Under these circumstances it would be unwise to recommend the use of different corrections for different grades of cows—in fact it is probable that no greater accuracy would be obtained by varying the corrections according to the total lactation yield, or according to the maximum yield; to give any probability of success it would have to be done according to the “persistency factor”—a difficult thing to estimate.

The work described in this Section seems to show that the corrections given in Table XIV are as accurate as possible for all these breeds and grades of cows, but that for those individuals giving a long level lactation curve, the figures are slightly too high for short S.P.'s and slightly too low for long S.P.'s.

#### SECTION B. THE EFFECT OF FOETAL GROWTH ON THE LACTATION CURVE.

*All Cows.* Section A of this Part has been devoted to an attempt to measure the total effect of the length of the S.P. on the lactation yield, and it has been pointed out that there is a probability that two effects

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are involved—the one that the length of the S.P. has on the length of the lactation, and a probable effect of foetal growth on the yield, as pregnancy progresses. The former of these is fairly straightforward and has already been dealt with, and it is the purpose of this Section to describe and measure the latter.

For this purpose average lactation curves have been worked out for cows served at different stages of the milking period. Of all the 3918 lactations in these data there were

163 with a S.P. of 180 days or more (Mean S.P. = 244.8 days)

714           ,,           from 40 to 59 days

495           ,,           ,,   20 to 39   ,,

These lactations were copied out again on to separate sheets and the average curves worked out; the principle adopted, then, was to treat the first group as empty cows, *i.e.* to assume that the rate of fall of that curve was that due to the progress of the lactation alone, and that the difference, from this rate, of the other two, was due to the superimposition of pregnancy, as a cause of rise or fall in yield. It will be seen that the long S.P. group were not empty for the whole of the time the others were in milk; the first served of these “empty” cows became

pregnant again in the  $\frac{180 - 4}{7} = 25\text{th}$  week of the lactation and the

average length of S.P. extended to the  $\frac{244.8 - 4}{7} = 34\text{th}$  week of the

lactation, whereas the other two groups remained in milk for 37 and 39 weeks respectively. It will be shown, however, that the effect with which we are concerned is only small for some time after service, so that a very minor error is introduced into this comparison by the above assumption.

The three lactation curves for the above groups are shown in Fig. 17, where a cross marks the time of service on each curve.

In comparing lactation curves account must be taken of the month in which the cows calved, for it has been seen (Part I) that the season of the year has a large influence on their shape. The lactations included in these particular curves were distributed fairly similarly as regards the month of calving, but more accuracy may be attained by allowing for any differences between their distributions in this respect; accordingly all of the curves were standardised to the mean curve for all cows, so that the seasonal effect might be eliminated. The method of doing this was described when the Penrith results were reported(5) and need not

be described again; it may, however, be said that experience shows that this method is very satisfactory.

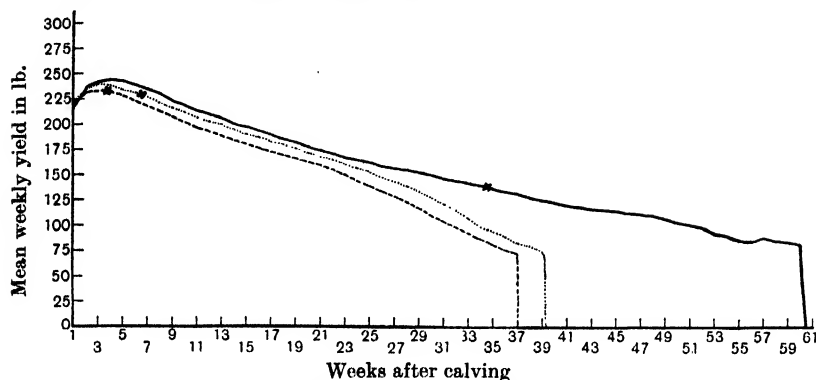


Fig. 17. Uncorrected mean lactation curves (all cows).

- S.P.=180 days and over (Mean=244.8 days); 163 lactations.  
 ..... S.P.=40-59 days; 714 lactations.  
 - - - - - S.P.=20-39 days; 495 lactations.

In the present instance, as would be expected from the above remarks, and from the fairly large numbers included, this standardisation had little effect on the relative shapes of the curves, so the standardised curves are not shown. From them was obtained the drop curve given in Fig. 18, which illustrates the effect of pregnancy on the milk flow.

In this figure the yield of "unserved" cows is represented by a horizontal line, and the broken line shows the mean distance, above or below it, of the served cows; the dotted line gives the corresponding result found from the Penrith data. In this case the weeks are measured from the time of service (approx. 4th and 7th week of lactation) and vertical lines show the limits of the interval of S.P. (20 days). As an example the first point corresponds to the 3rd week before service; to obtain this the difference between the 1st week's yield (of the lactation) of cows served 20-39 days after calving and the 1st week's yield of "unserved" cows was averaged with the difference between the 4th week's yield of cows served 40-59 days after calving and the 4th week's yield of "unserved" cows.

Taking the drop curve for Norfolk first, we see that there is a noticeable decline in milk yield as the curve enters the service interval; the incidence of heat appears to have a very variable effect on milk secretion, but, unless this fall be regarded as a chance result, it seems that, on the average, the occurrence of heat is associated with a slight fall in yield.

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The decline is continued throughout the service interval and after pregnancy has commenced the yield settles down at a definitely lower level than before; this reduction amounts to less than a gallon a week, but appears to be quite definite and continuous for the first 20 weeks

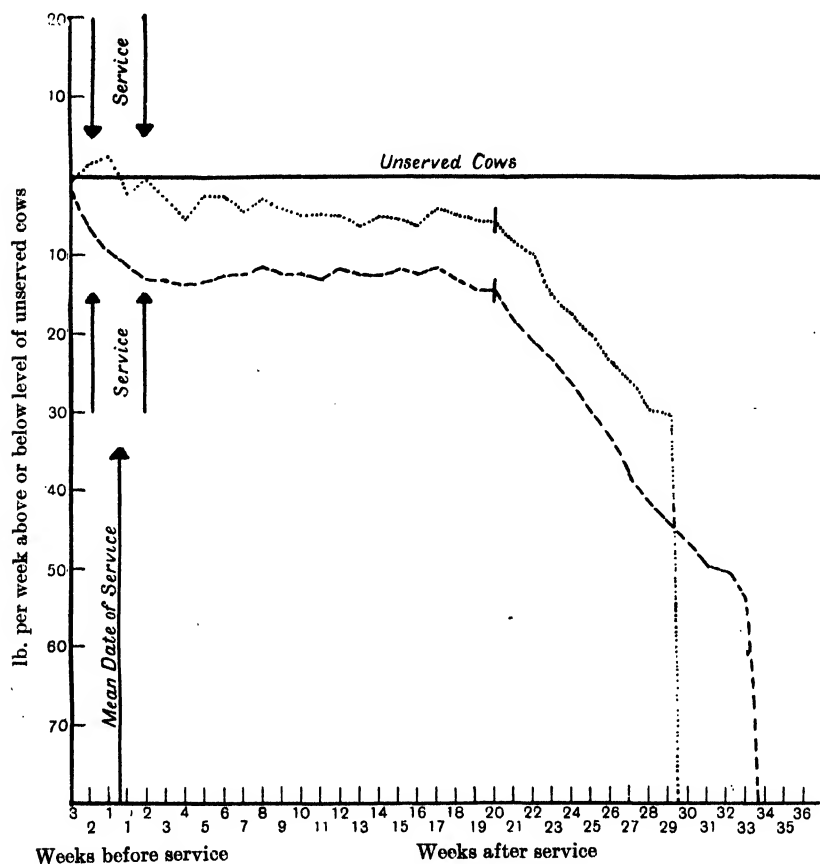


Fig. 18. Effect of foetal growth on milk yield.

----- Norfolk (all cows).

..... Penrith (all cows).

of pregnancy, when the fall suddenly becomes very steep (the 20th week of pregnancy is marked by a short vertical stroke on the curve).

The Penrith result is very similar, but one point must be mentioned: owing to the longer S.P.'s in that district the curves for served cows were for those whose S.P.'s fell within the intervals 40-59 days and 60-79 days, and consequently the actual yields involved in the comparison were considerably less; this may explain the absence of fall at

the onset of heat in this case. Otherwise the figures agree admirably and it should be noticed that the change from the slow to the rapid fall occurs at exactly the same point—the 20th week of pregnancy.

In Fig. 19 these weekly drops are shown as percentages of the yield of “unserved” cows throughout; this seems to be a better way of

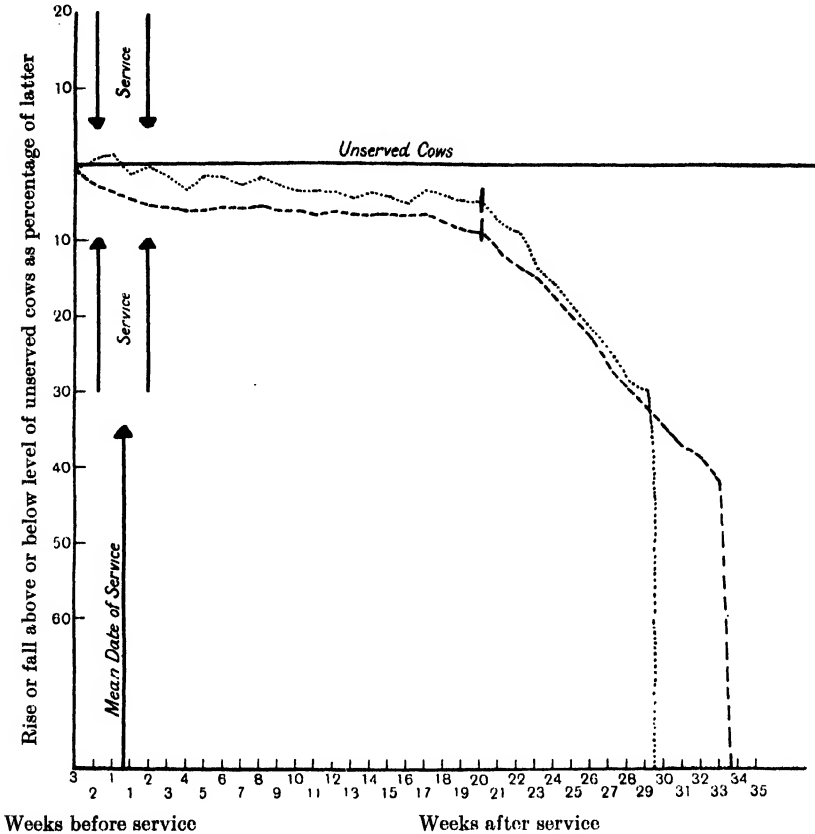


Fig. 19. Effect of foetal growth on milk yield.

----- Norfolk (all cows).

..... Penrith (all cows).

comparison, for allowance is thus made for the fall in yield as the lactation advances, a difference of 20 lb. being of greater import when the yields are round about 150 lb. a week, than when they are in the neighbourhood of 250 lb. a week. In the writer's view lactation curves are parallel when the ratio between the yields remains constant (*i.e.* curves that are parallel when drawn on a logarithmic scale) and not when



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the difference between them does so—parallelism, then, has a definite meaning both from the biometrical and physiological standpoint.

By this method of representation the two curves are brought nearer together and it appears that the fall in the first 20 weeks is in approximately the same proportion in Norfolk as in Penrith; it will be seen, too, that this fall is now progressive, the loss of milk being greater, for instance, at the 15th week after service than at the 5th week, when it is expressed as a percentage of the yield of empty cows. In Fig. 18 the reverse appears, the drop curves tending to approach the level of unserved cows during this period—clearly this is due to the progress of the lactation and the associated fact that all the yields are falling; the other figure demonstrates, though, the more important fact to the physiologist, that the *proportionate* decrease due to foetal growth is progressive during the first 20 weeks after service.

Fig. 19 provides a rough means of testing the corrections for different lengths of S.P., given in Section A; for this purpose the standardised curve, used above, for all cows with S.P.'s of over 180 days was employed. From this were calculated the lactation yields to be expected for different lengths of S.P., by subtracting, week by week, the percentage here found, and assuming the lactation to end in each case 33 weeks after service (it will be realised that this assumption is not quite justified).

The method was, perhaps, rough, but it was thought that it should give results approximating to those already found, if the latter were truly representative of this function. The results were surprisingly close, as is shown in Table XIX.

Table XIX.

S.P. Days	From drop percentages		Percentage corrections from correlation between lactation yield and length of S.P. (Table XIV)	
	Calculated lactation yield (lb.)	Percentage correction		
			1st calvers	Others
9.5	5549	+27.5	+28.2	+33.9
29.5	5996	+18.0	+18.4	+21.3
49.5	6421	+10.2	+10.6	+11.9
69.5	6821	+ 3.7	+ 4.2	+ 4.6
109.5	7508	- 5.8	- 5.5	- 5.9
129.5	7812	- 9.4	- 9.2	- 9.7
149.5	8088	-12.5	-12.4	-12.9
.85	7076	—	—	—

In the first column of this table is given the lactation yield, calculated as described, for the S.P. on the left; next follows the correction obtained from this (e.g.  $\frac{7076}{5549} = 1.275$ ), and the last two columns give the corre-

sponding corrections given in Table XIV. The agreement is very good, which indicates that the corrections given before were not subject to errors arising from the unequal distribution of good and bad cows over the range of S.P.; it will also be noticed that these figures conform much more closely to the logarithmic than to the linear type of corrections.

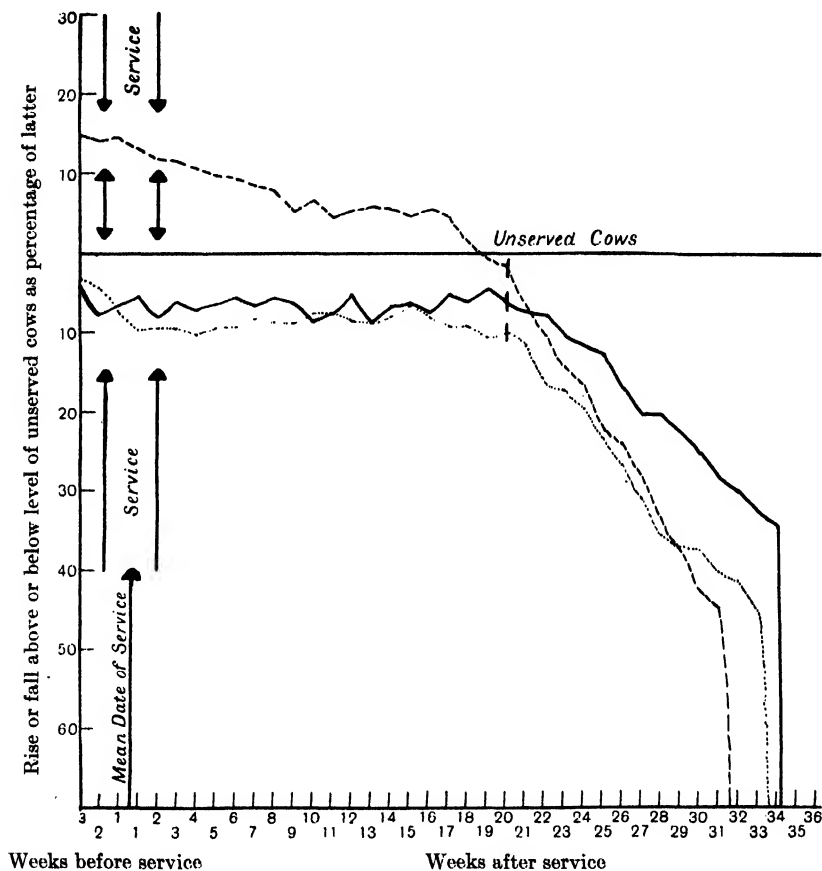


Fig. 20. Effect of foetal growth on milk yield.

Red Polls ———

Lincoln Reds - - - - -

Friesians . . . . .

*Breeds.* Average curves have been drawn up for Red Polls, Lincoln Reds and Friesians in the same way; the number of lactations available were:

	S.P. = 180 days or more	S.P. = 40-59 days	S.P. = 20-39 days
Red Polls	33	146	93
Lincoln Reds	30	86	79
Friesians	36	78	45

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The curves were standardised to the mean curves for each breed—*i.e.* to standardise a Red Poll curve the 12 monthly curves for Red Polls were used, and so on. The three percentage drop curves obtained as above are shown in Fig. 20.

It will be seen that the numbers involved in the curves for empty cows were small, and although sufficient to give reasonably smooth lactation curves were insufficient to ensure that the cows included should approximate to the mean for the breed; this has led to the drop curves starting either above or below the level of empty cows; they, however, still suffice as a means of allowing for the progress of the lactation.

Friesians follow the results for All Cows exactly; there is a noticeable decline as the curve enters the service interval, and it varies round a slightly lower level for the first 20 weeks of pregnancy, and at that exact point there is an abrupt falling off.

Red Polls are not quite similar; the percentage “drops” for the three weeks before service are 4.08, 7.71 and 6.76 (mean = 6.18) whilst the mean of those for weeks 1–20 of pregnancy is  $6.56 \pm 0.155$ —*i.e.* the level is slightly lower, but the difference does not approach significance; neither is the break at the 20th week of pregnancy a clear cut one—it is at about this point that the drying off process commences, but the curve is more rounded than normal.

With Lincoln Reds the reverse appears; here the fall following service is marked and progressive during the first part of pregnancy, and the 17th week shows a fairly abrupt change to the rapid fall. The effect is therefore considerably greater in the case of this breed, and this carries us a step further in the explanation of the comparatively steep mean curve for Lincoln Reds (Part I); empty Lincoln Reds appear to show persistency, at least as well as other breeds, for, from the corrected curves for the unserved groups used here, we have:

	Maximum weekly yield lb.	Yield during 40th week of lactation lb.
Red Polls	230.3	104.5
Lincoln Reds	215.3	119.4
Friesians	279.2	148.9

where it appears that the yield of empty Lincoln Reds is maintained quite as well as that of empty Friesians and considerably better than that of empty Red Polls.

Whilst then, broadly speaking, the effect of foetal growth on milk yield is of the same type in these three breeds, there appear to be some modifications; Friesians conform to the variation found with All Cows,

but Red Polls show a smaller effect, and with Lincoln Reds the fall is markedly accentuated throughout. These observations provide no adequate explanation of the fact that the regression of lactation yield on length of S.P. was found to be linear for Friesians and Lincoln Reds, and it is believed that that result arose, as a result of the limited number of records available.

#### HIGH AND LOW YIELDERS.

Drop curves have been obtained in the same way from the two groups here described as High and Low (Total) Yielders; the numbers of lactations, included in the lactation curves that gave these figures, were as follows:

	S.P. 160 days or more	S.P. 40-59 days	S.P. 20-39 days
High (total) yielders	40	133	83
Low (total) yielders	30	120	100

It will be noticed that the lower limit for S.P. had to be taken at 160 days to give enough lactations for the "unserved curves"; the drop curves obtained from these lactations are given in Figs. 21 and 22, the former showing the difference between pregnant and empty cows in pounds, and the latter this difference as a percentage of the yield of empty cows.

Again the numbers included in the unserved groups were insufficient to give a mean level of yield approximating to that of the others, but the rates of fall of the two curves are obviously more similar in Fig. 22 than in Fig. 21; from the 20th week onwards this is very plain and for weeks 1-20 we have the following:

	Mean distance below level of unserved cows for 3 weeks before service		Further drop in weeks 1-20 of pregnancy	
	In pounds	As percentage	In pounds	As percentage
High (total) yielders	- 5.33	- 2.09	+ 7.58 $\pm$ 0.59	+ 3.14 $\pm$ 0.28
Low (total) yielders	+ 21.10	+ 9.33	+ 0.44 $\pm$ 0.50	+ 3.19 $\pm$ 0.43
	Difference		7.14 $\pm$ 0.77	0.05 $\pm$ 0.51

The drop in the first 20 weeks of pregnancy is noticeably and significantly greater, in actual pounds, for high yielders, but exactly the same when expressed as a percentage of the yield. It is surprising to note in the case of the low yielders the very different result obtained by the percentage method as against the other; the actual distance below the level of unserved cows is less for weeks 6-16 of pregnancy than in the three weeks before service, whereas Fig. 22 shows that (except for the 8th week) the yield of pregnant cows is never such a high percentage of that of empty ones as before service. Here we have a case of high

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and low yielding sub-groups among the low yielders, which means that the curves will approach each other as the yield falls.

From the above it seems safe to conclude that high and low yielders show the same proportionate diminution of yield throughout pregnancy, which means that the better the cow the greater the number of actual pounds in the drop caused.

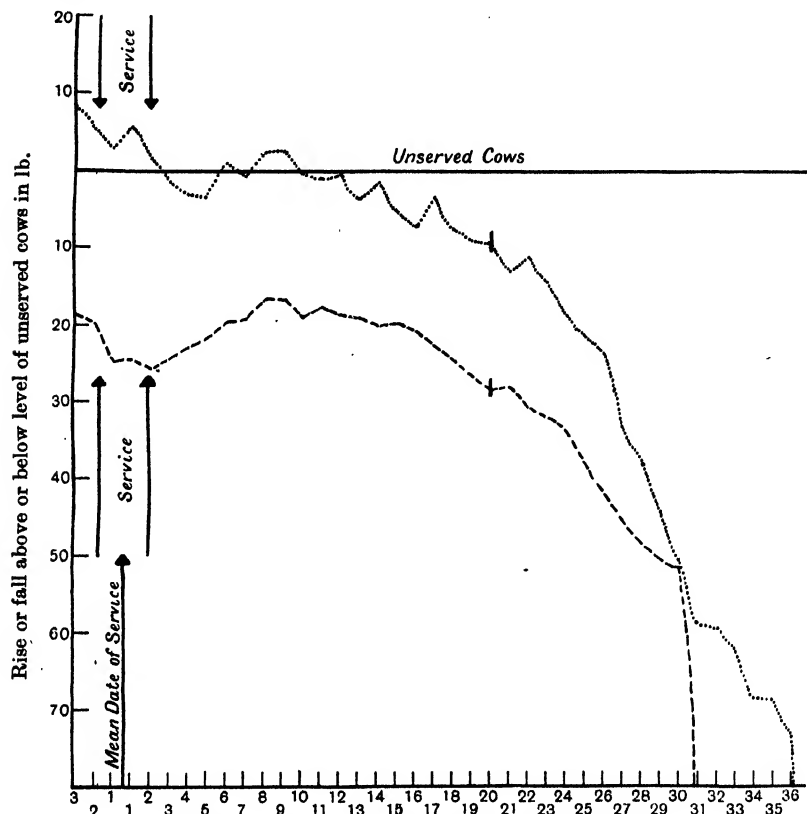


Fig. 21. Effect of foetal growth on milk yield.

..... High (total) yielders.

----- Low (total) yielders.

Before concluding this Section it would be advisable to summarise the results described and to attempt to interpret their bearing on the physiological aspect of the question. Three points arise:

(A) There seems to be a slight decrease of yield associated with the onset of heat; this is very variable, and might be attributed to the excitability generated by sexual desire.

(B) Immediately after service the yield settles down on a slightly

lower level than before, and continues on this level for about 20 weeks; when expressed as a proportion of the yield of empty cows this initial drop appears to be slightly progressive during the first 20 weeks.

(C) At or about the 20th week of pregnancy a sudden and continuous fall sets in.

The causes of (B) and (C) are debatable. When the Penrith data were reported the following conclusion was reached—"that the slight

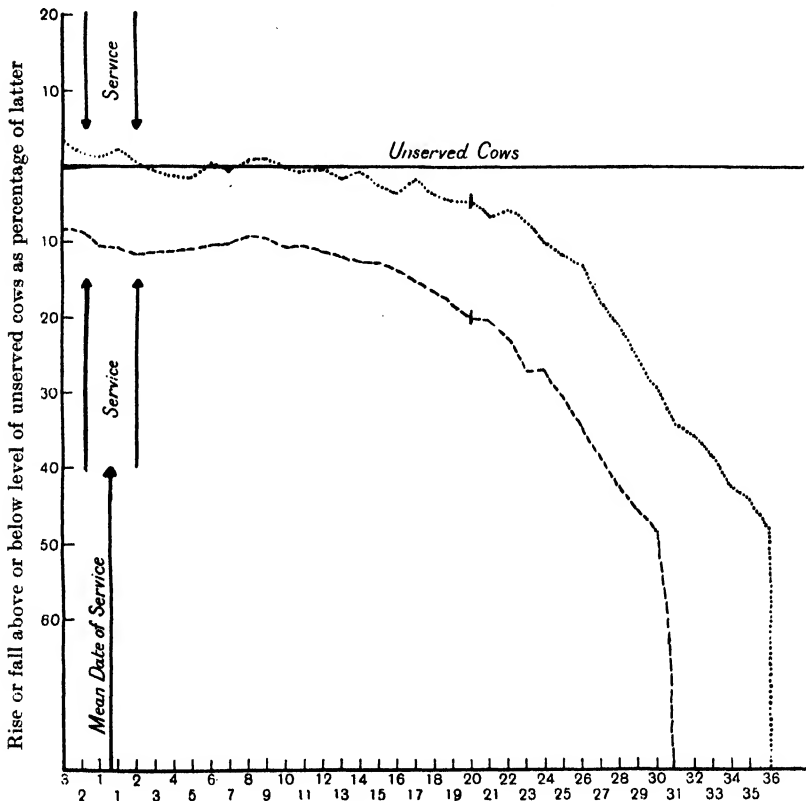


Fig. 22. Effect of foetal growth on milk yield.

fall immediately after service is due to natural causes resulting from the growth of the uterus and its contents, and that the definite drying off at 20 weeks after service is brought about by the internal secretions from the reproductive organs initiating an anabolic phase of growth in the mammary gland." This accords with the above results for All Cows, since the effect there is exactly similar to that for Penrith, but the division of the data, into breeds and high and low yielding individuals,

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has shown minor modifications, which can hardly be ascribed to chance, and which suggest that the effect is not quite so clear cut as might have been supposed. Thus Red Polls show hardly any fall in early pregnancy whilst Lincoln Reds show a greatly abnormal one; low yielders show very little initial drop in pounds, but when this drop is expressed as a proportion of their assumed yield if empty, it is in surprising agreement with that for high yielders. The incidence of the break also shows some variation; with Lincoln Reds it plainly occurs at the 17th or 18th week, whereas with Red Polls it is at about the 20th, but is not so apparent, the curve being more rounded; with high yielders it occurs at the 22nd week (after a small preliminary fall), whereas with low yielders it commences at the 17th week.

Are these results compatible with the above conclusion? Supposing that the initial drop were due to the demands made by the foetus, etc., it would, perhaps, be expected that it would be slightly greater with high yielders than low yielders in actual pounds, though if the two groups were fed according to yield, the lost milk should be the same; we have found, here, that it is considerably greater with high yielders, and it can hardly be due to chance that the proportionate drop has been found to be so nearly the same. This comparison seems to suggest that it is not a nutritional effect, but a more purely physiological one; mammary growth is believed to occur in a series of cycles; during a heifer's first pregnancy it is very pronounced, but during each succeeding gestation further growth takes place, either resulting in the addition of more mammary tissue or in the "recommissioning" (as it has been called) of the alveoli, or both. If mammary growth and recommissioning were responsible for the initial drop in pregnancy, this result for high and low yielders would be expected.

Against this view might be urged the fact that the Lincoln Red calf is a large one at birth, even in proportion to the weight of its dam, and that this would explain the larger drop for this breed. Eckles(2), however, has shown that the nutrients required for the development of the foetus are almost insignificant compared to those necessary for milk production (1.9 per cent. of the annual milk yield) and it is also known that during the first half of pregnancy the foetus and its membranes consist very largely of water. The objection is further discounted by the fact that the Friesian calf is also large at birth (8 per cent. of the weight of its dam, as against 6 per cent. in the case of the Dairy Shorthorn(3)), whereas these figures show that there is no abnormal initial fall in this breed during pregnancy.

On the whole the evidence seems rather to contradict the first part of the conclusion drawn from the Penrith data and to suggest a definite physiological factor (probably mammary growth) as being responsible for the initial drop after service, and it would also seem probable that a similar cause is responsible for the further drop in the second half of pregnancy.

We have seen that the incidence of this large fall, which we must now regard as being due to a sudden change from a slow to a comparatively rapid rate of mammary growth (or whatever the factor may be), is susceptible of some variation, but it is quite well marked, and occurs somewhere about the 20th week. In our present state of knowledge no explanation of this change is available, but it is known that it is at exactly the same stage of pregnancy, with cows and other animals, that other changes take place. Mention might be made of the work of Woodman and Hammond (7), which has been followed up by Asdell (1), on the composition of the secretions obtained from heifer's udders during their first pregnancy; at the 20th week this secretion suddenly becomes honey-like in consistency and the percentage of globulin rises to the neighbourhood of 30, the fluid then obtained giving a mixture, with normal milk, resembling colostrum.

The quest for the factors responsible for these variations of yield associated with pregnancy, seems now to pass from the biometrician to the experimental physiologist, to whom might especially be commended the difference here noted between Red Polls and Lincoln Reds—these two breeds seem always, in their behaviour, to lie slightly off the normal, and invariably on opposite sides.

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# A NOTE ON THE PRODUCTION OF PREMATURE SPROUTING IN THE POTATO, AND ITS APPLICATION TO THE STUDY OF VIRUS DISEASES.

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WORKERS on Virus Diseases of the Potato labour under a disadvantage inasmuch as they are frequently confronted with the difficulty of obtaining positive results of infection in one and the same season. Moreover, tubers resulting from a plant which has been artificially infected in one season, retain the secret of the success or otherwise of the operation until adult plants have been grown from them in the succeeding year. Any method which will sensibly curtail this long waiting period is welcome. As long ago as 1788, Joseph Webb<sup>1</sup>, writing on "Curl" in potatoes, recommended that a sample of the seed-tubers intended for planting in the following season should be planted in a hot-bed before Christmas, and that if 2 per cent. or more of the plants exhibited Curl, the stock should be destroyed. The success of any attempt to grow potatoes out of season depends on the possibility of breaking down the dormant period of the tuber. This dormant period is the more readily cut short the nearer the tuber is to its natural sprouting period, so that in most cases there is no particular difficulty in growing plants from tubers under glass in the early part of the new year. The problem which interests the worker on Virus Diseases is to raise plants at such a time that he may by grafting or other means obtain a positive reaction in the green plant by at least the middle of March, and by so doing obtain information which will guide him in dealing with the material destined for growth in his field plots in the summer of the same year. Denny's<sup>2</sup> recent papers on the abolition of the latent period by means of chemicals appeared to indicate the path along which a solution of the problem might be secured. Appleman<sup>3</sup> had previously shown that the latent period might be abruptly cut short by removing the skin of the tubers

<sup>1</sup> Webb, Joseph (1788). *Bath Papers*, Vol. 3, p. 306.

<sup>2</sup> Denny, F. E. (1926). Effect of Thiourea upon bud inhibition, etc. *Contributions from Boyce Thompson Institute*, Vol. 1, No. 3, p. 154. Second report on the use of Chemicals for hastening the sprouting of dormant Potato Tubers. *Ibid.* Vol. 1, p. 169.

<sup>3</sup> Appleman, C. (1914). Study of Rest Period in Potato Tubers. *Maryland Agric. Exp. Stn. Bull.* 183.

and that cut portions of tubers reacted more readily than whole ones. Denny tested with great care a number of reagents and of these found three—two were solutions of sodium and potassium thiocyanate ( $\text{NaKCNS}$ ,  $\text{KCNS}$ ), the other a solution of thiourea ( $\text{NH}_2\text{CSNH}_2$ )—to be amongst the most active. These two solutions were employed by the writer. The following notes on the small scale trials of 1926-7 would, however, not be published now but for the fact that in their performance an effective and very simple method of obtaining early sprouting manifested itself, and one moreover which allowed of grafting and positive results being obtained in regard to virus infections before the middle of March.

The trials made may be briefly summarised:

*Trial I.* Oct. 9, 1926. Single tubers of Great Scot harvested on August 28 and President harvested on September 16, were split into three parts: one part was soaked in a solution of potassium and sodium thiocyanate, a second in a solution of thiourea, and a third in water. Five such series were made: the period of immersion and the strength of the solutions are shown in Table I. The length of the sprouts, in inches, is the maximum length attained on each one-third tuber.

Table I.

Variety	Condition of tuber	1 hour			2 hours			11 hours			1 hour			11 hours		
		Water	Thiourea 1 %	Thiocyanate 1 %	Water	Thiourea 1 %	Thiocyanate 1 %	Water	Thiourea 1 %	Thiocyanate 1 %	Water	Thiourea 2 %	Thiocyanate 2 %	Water	Thiourea 2 %	Thiocyanate 2 %
Great Scot	Cut	ins.	ins.	ins.	ins.	ins.	ins.	ins.	ins.	ins.	ins.	ins.	ins.	ins.	ins.	ins.
President	1/3	0.75	0.5	0	0.75	0.5	0.15	0.6	0.75	0	0.75	0.15	0	0.5	0	0
	„	0.5	0	0	0.75	0	0	0.5	0	0	0.5	0	0	0.5	0	0

Date of immersion October 9, 1926.

After immersion the tuber pieces were rinsed and dried, and kept in a cool room during the winter. Inspection from time to time showed that the cut surfaces rapidly suberized, that the control pieces (water immersion only) remained alive and normal, but that the steeped pieces were, as time went on, to a greater or lesser extent damaged, becoming dried up and rotten. The results recorded in Table I are those observed on June 24, 1927, and differ but little from those observed in April.

Failure of the sprouts to grow seems to be the direct result of the action of the chemicals. It should, however, be remembered that the tubers were kept dry for eight months and for more than half that time in a cool room, conditions obviously not conducive to sprout formation.

If no beneficial but rather harmful effects are to be observed from the treatment, it is to be recognised that the two varieties react very differently to the treatment and that Great Scot is not only more resistant to the poisonous effects of the chemicals employed, but that it is inherently an earlier and freer-sprouting variety than is President.

*Trial II.* The varieties used were King Edward and Arran Chief; the solutions the same as in Trial I; the strength and times of immersion were varied. After immersion the tubers were placed in trays, covered with sand, and kept moist in a glasshouse at an average temperature of 11° C. The number of tubers actually under test in each case being few, a detailed statement of the results is of no real value, so that in Table II the average length of sprouts resulting from the various immersions in either solution is given and compared with the controls which were immersed in tap water for equal lengths of time.

Table II.

Variety	Condition	No. of tubers	Controls ins.	No. of tubers	Thiocyanate solution ins.	No. of tubers	Thiourea solution ins.
King Edward	Whole	4	0.12	2	0.06	2	0.4
"	Half	4	1.5	4	1.5	4	1.6
Arran Chief	Whole	4	0.5	2	0.5	2	0.9
"	Half	4	1.8	3	2.0	3	2.0

The tubers were immersed on December 3, 1926, and examined on January 3, 1927.

In this trial, as in the former one, is found an indication of a varietal difference in regard to sprout stimulation by the two solutions used, but more striking, and despite the small number of tubers employed, obviously significant is the fact that the mere splitting of the tubers immediately before immersion, and imbedding in moist sand, stimulates the growth of the sprouts to an extraordinary degree far surpassing the effects induced by either of the solutions employed.

*Trial III.* In this trial the same two solutions, viz. a 3 per cent. mixture of equal parts of sodium and potassium thiocyanate and a 3 per cent. solution of thiourea, were used as stimulants. The tubers treated were of the varieties Arran Chief, King Edward, and President. Whole and cut tubers were employed as before, and in addition "cores" of the two earlier varieties, obtained by extracting from tubers of each a 2-3 inch long core with an eye at one end by means of a cork-borer 13 mm. in diameter. After immersion, all the tubers were placed in trays of moist sand and maintained at an average temperature of 11° C. in the glasshouse. The trial was begun on January 4 and the sprouts

were measured on February 5. The controls were placed in water for equivalent periods and then kept, one set in moist sand, the other in dry sand, in the same glasshouse. The results are shown in Table III.

Table III.

Variety	Con- dition	No. used		No. used		No. used		No. used		No. used		No. used		No. used	
		Control wet	ins.	Control dry	ins.	Thiocyanate 3 % 1 hr.	ins.	Thiocyanate 3 % 2 hrs.	ins.	Thiourea 3 % 1 hr.	ins.	Thiourea 3 % 1 hr.	ins.	Thiocyanate 3 % 1½ hrs.	ins.
King Edward	Whole	10	0.8	12	0.6	4	0.9	4	1.0	4	0.6	—	—	4	0.8
	Half	12	1.0	10	0.4	4	1.6	4	1.8	4	1.0	—	—	4	1.3
"	Core	6	2.2	—	—	—	—	—	—	—	—	3	0.33	—	—
Arran Chief	Whole	10	1.5	12	0.5	4	1.2	4	1.4	4	1.0	—	—	4	0.9
	Half	12	1.8	10	0.8	4	2.8	4	2.0	4	2.0	—	—	4	2.0
"	Core	6	1.5	—	—	—	—	—	—	—	—	3	1.2	—	—
President	Whole	7	0.0	—	—	—	—	4	0.1	—	—	—	—	8	0.4
	Half	10	0.0	—	—	—	—	4	0.4	—	—	—	—	10	0.6

The pronounced effect of merely cutting the tuber is again demonstrated and in the case of the "cores" of King Edward it results in sprouts nearly three times the average length of those growing on the "whole" tuber controls. The influence of the thiourea solution on the cores is to inhibit to a greater or less extent their growth. A similar effect of thiourea as compared with thiocyanate is to be observed with regard to both "whole" and half tubers of King Edward and Arran Chief, and in no case is the effect of the chemical stimulants, when compared with the controls in moist sand, sufficient to be of any practical value.

President, a variety which has a long and highly resistant resting period, only shows a demonstrable reaction to stimulation when cut, exhibiting though in a lesser degree the effect which has been previously noted in regard to the varieties King Edward and Arran Chief.

Denny pointed out that solutions of thiourea, besides stimulating sprout production, abolished the dominance of the apical over the lateral eyes, and at the same time brought about the production of multiple sprouts from the same eye.

The first effect, the abolition of the dominance of the apical eye by thiourea solution, was found to be more or less general in the varieties King Edward, Arran Chief, and Great Scot, though in those trials it would be more correct to describe its action as a partial rather than a complete one. The second effect, the production of multiple sprouts,

was found to be true in all the varieties under examination and particularly so for Arran Chief.

In Table IV the average of the maximum number of sprouts from any one eye on a tuber or tuber piece is shown.

Table IV.

Variety	Condition	No. used	Controls No. of sprouts per eye	No. used	Thio- cyanate No. of sprouts per eye	No. used	Thiourea No. of sprouts per eye
King Edward	Whole	14	1.1	10	1.0	10	1.2
"	Half	14	1.0	12	1.3	12	1.5
"	Core	6	1.0	—	—	3	3.0
Arran Chief	Whole	14	1.7	10	1.6	10	3.0
"	Half	14	1.1	11	1.3	11	2.5
"	Core	6	1.0	—	—	3	3.0
President	Whole	7	1.0	4	1.0	8	1.6
"	Half	10	1.0	4	1.0	10	2.0

From the table it appears that thiourea does lead to the production of multiple sprouts in a striking manner, and that merely cutting the tuber either in half or by the extreme method of making "cores," does not in itself have an influence of a like kind.

The sprouts thus obtained, and in particular those derived from the "cores," were removed with or without a fragment of tuber, but wherever possible with as many rootlets as could be detached, during the early part of February. The sprouts were then grafted with sprouts from other tubers obtained in like fashion but which themselves had originated from plants which in the previous season were known or suspected to be infected with one or another Virus Disease. The grafted sprouts were then potted up in "64" pots and kept in the dark for a few days. The results were in general highly successful; occasionally when a sprout but 1-inch long was used as stock, the resulting plant was all scion, but if the graft was not made too low, a branch shoot from the stock would grow up alongside the scion. Early in March the grafted plants were from 6-12 inches in height and in the trials under discussion displayed in the stock branches the diseases streak, crinkle, simple mosaic or aucuba, with which they had been infected by means of the scion.

#### CONCLUSIONS.

Whilst the chemical stimulants used do certainly aid in the removal of the latent period of the potato tuber, cutting of the tuber—especially by the extreme method of making "cores"—leads to a rapid and early formation of young and strong sprouts.

Varieties react differently towards the two chemical stimulants employed. A thiourea 3 per cent. solution appears to be more inclined to injure the tuber piece than is a 3 per cent. solution of sodium-potassium thiocyanate.

Thiourea solution causes more sprouts to arise from a single eye than does either thiocyanate solution or the absence of any special treatment.

Different varieties, apart from any treatment, have very different latent periods, and sprout with varying degrees of speed.

By means of sprout-grafting during the winter months, positive results of Virus Disease infection may be obtained early in March in a glasshouse with an average temperature of 11° C.

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# LATERITE AND LATERITIC SOILS IN SIERRA LEONE.

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(With Plates III and IV.)

IN a previous communication<sup>(1)</sup>, a description was given of the various soil types of Sierra Leone. These were described as laterites and lateritic sands, sandy loams, etc., emphasis being laid on the fact that all the soils examined had a low silica/alumina ratio in the clay fraction. Since the publication referred to, further investigations have been carried out relating to the formation of laterite in Sierra Leone, and the results of these investigations are embodied in this paper.

*What is laterite?* Before attempting to define the term "laterite" it is perhaps as well to review briefly the history of work on laterite<sup>1</sup>. A very good account of this is given by Shantz and Marbut<sup>(2)</sup> and the salient points are as follows. In many tropical countries the soils are distinguished by an ever prevalent red colour, and in many of these soils there is a varying proportion of concretionary masses containing a high percentage of iron. The name laterite was first given by Buchanan in 1807 to the red coloured earth used by the natives of India in building houses, on account of the ease with which blocks of hardened material could be formed by cutting the soft or slightly consolidated soil and exposing them to the air, when, on drying, they became sufficiently hard to be used as a substitute for brick. Subsequently until the beginning of this century it became the practice for travellers in the tropics to refer to all red soil as laterite. This was due mainly to the lack of a chemical knowledge of these soils and a lack of agreement among scientific workers as to what characteristics denoted laterite.

Two kinds of laterite were recognised, detrital and residual laterite. Detrital laterite is that laterite which has been removed by erosion and accumulated by deposition in another place, while residual laterite is that laterite which is formed *in situ*, and is usually characterised by the presence of iron oxide concretions. It was often stated that residual laterite constituted true laterite, while detrital laterite was alluvium derived from lateritised rocks.

<sup>1</sup> Fox's recent bibliography (*Mem. Geol. Surv. India*, 49, Pt. I, 1923) will help readers who wish to go further into this subject.

Bauer (3, 4), in his work on the soils of the Seychelle Islands, examined samples collected by Brauer of all stages in the weathering of the unchanged granite and diorite, to the final product identified by Brauer on the basis of its physical properties as laterite. He showed that the completely weathered product retained the structure of the parent rock; that the alumino-silicate minerals changed entirely to a mass of colourless flakes showing well defined polarisation under the microscope; that the iron became segregated into cracks with more or less impregnation of the flaky material, and that the places formerly occupied by minerals were filled to a large extent by compounds of iron; that the quartz of the parent rock remained in the laterite without having suffered any recognisable change; that no essential difference can be found in the weathered material whether from granite or diorite; that the essential constituent of laterite consisted of the colourless flaky mass and that its formation was essentially independent of the character of the parent rock.

Chemical analyses showed that, after the removal of the quartz grains, the laterite from granite consisted of 60 per cent. alumina, 9 per cent. iron and 29 per cent. of combined water. The laterite from diorite contained 52 per cent. alumina, 21 per cent. iron oxide and 27 per cent. water. The iron, like the quartz, is variable and is best considered as the unessential constituent. The essential constituent, aluminium hydroxide, exists in a crystalline state and considerations of the possible combinations of alumina and water led Bauer to the conclusion that the characteristic mineral present is hydrargillite or gibbsite ( $\text{Al}_2\text{O}_3 \cdot 3\text{H}_2\text{O}$ ). He considered that except for impurities present laterite is identical with the bauxite of the Northern Hemisphere. Papers by H. and F. J. Warth<sup>(5)</sup> and T. H. Holland<sup>(6)</sup> showed that many of the "laterite" soils of India were of a similar composition to the products from the Seychelles examined by Bauer. Other papers by J. B. Scrivenor<sup>(7)</sup>, J. B. Harrison<sup>(8)</sup>, T. Crook<sup>(9)</sup> and M. Maclaren<sup>(10)</sup> showed that material previously described as laterite did not contain significant quantities of the oxides of aluminium.

Fermor<sup>(11)</sup> in 1911 published a paper in which he discussed the definitions of the term laterite. He proposed that the term "laterite" should be restricted to soils containing from 90 to 100 per cent. of lateritic constituents (these he defined as the oxides of alumina, iron, titanium and manganese more or less hydrated); that soils containing from 50 to 90 per cent. of laterite constituents should be called siliceous laterites or lithomargic laterites; that those containing from 25 to 50



per cent. of these constituents be called lateritic lithomarge or lateritic soil, while those with less than 25 per cent. should receive no designation based on the presence of lateritic constituents.

Lacroix<sup>(12)</sup> differed from Fermor in some of the details of his definition, particularly with reference to the form in which the aluminium hydroxide occurs, but agreed in the greater part of his classification according to the percentage of hydroxides present.

One of the latest contributions is that of J. M. Campbell<sup>(13)</sup> who is concerned primarily with the processes of laterite development. He separates the processes of rock decomposition in the tropics into two groups, *alteration* and *weathering*. The former takes place within the zone of permanent saturation (by water) and consists of the elimination of iron and the conversion of alkaline silicates of alumina into hydrous silicate of alumina. The result is the formation in this zone of a bleached mass of hydrous silicate of alumina. In no case is aluminium hydroxide produced in this zone.

True weathering takes place only within the zone of intermittent saturation, and it is in this zone that the formation of laterite takes place. This process cannot operate directly on the parent rock but only on rock which has become kaolinised during its passage through a zone of permanent saturation. According to Campbell laterisation cannot take place directly on the parent rock; nothing could be more misleading than to suppose that a highly evolved laterite comes into existence by the re-arrangement, alteration, weathering or metasomatic replacement of the constituent minerals of a crystalline rock. Laterite is therefore a product of changes peculiar to the tropics acting on material which has previously been changed by the processes identical with those usually operating in temperate and cool climates.

Shantz and Marbut (*loc. cit.*) accept the general classification of Fermor and Lacroix, and they define laterite and lateritic red loams as follows: "Laterite" will be used to include those soils whose composition is the same as Fermor's first group or those containing from 90 to 100 per cent. of lateritic constituents, while all soils containing from 25 to 90 per cent. of lateritic constituents will be described as "lateritic red loams."

The definition of Fermor and Shantz and Marbut place the terms "laterite" and "lateritic" on a definite chemical basis, and the next thing to consider is whether this basis is satisfactory. We do not think the definition is adequate, since by including iron oxide as a lateritic constituent it would place in the same class such diverse soils as some

parts of the ferruginous outcrops of the Middle Lias formation in Oxfordshire, and the ordinary Sierra Leone soils (hitherto called laterite), and in the same category such diverse substances as the low grade bauxite (51 per cent.  $\text{Al}_2\text{O}_3$ ) at Wilberforce (Sierra Leone) and the haematite deposits (65 per cent.  $\text{Fe}_2\text{O}_3$ ) of the Marampa Hills (Sierra Leone). It therefore appears that the inclusion of iron oxide as a lateritic constituent is not desirable since the proportion of iron oxide may be high, irrespective of the relative proportions of silica and alumina present. This coincides with the views expressed by Bauer (*supra*).

The method of defining laterite by the proportion of lateritic constituents present in the whole soil is open to some objection on the ground of the difficulty of arriving at an estimate of the exact proportions of the lateritic constituents present. To begin with, it is difficult to take a small representative sample of a soil where the stones and gravel form over 40 per cent. of the soil and where these particles may vary in size from one millimetre to one metre (and often more) in diameter. Moreover, it is extremely difficult to remove the quartz quantitatively (only combined silica is considered when calculating the proportion of lateritic constituents present) both in the finer fractions, where mechanical separation is practically impossible, and often in the larger fractions, such as lateritic concretions, where quartz particles may be embedded and are reduced to powder when the concretions are pulverised for analysis. The determination of the amount of combined silica present in the whole soil is therefore extremely difficult.

From the point of view of the soil chemist, as well as that of the geologist, the composition of the clay fraction is probably of the greatest interest in determining the reactions of the soil, and we think that for agricultural purposes it may be better to base our classification of lateritic soils on the composition of the clay fraction rather than that of the whole soil, the larger fractions of which are from an agricultural chemical point of view, comparatively inert. It is of course desirable that our definitions of laterite should agree as closely as possible with those of geologists, and we think that a classification of soil based on the composition of the clay fraction will not conflict seriously with geological views but will on the other hand give information more easily obtained and more obviously significant than that obtained by an analysis of the whole soil. We have examined a number of soil fractions of lateritic soils, and before entering further into a discussion of laterite we will describe our results.

*Red "laterite" soil from the Norite hills of the Colony of Sierra Leone.*

A short geological description of these hills is given by F. Dixey (14):

The norite complex forms the mountain mass which, together with a narrow coastal plain of Pleistocene sediments, makes up the Sierra Leone Peninsula. The oldest and most important member of the complex is an olivine-norite, which was intruded in the form of a huge stock. This norite is thus very different from other well-known norites, which occur chiefly as laccolites or as special phases of larger masses of different composition. It does not appear to possess any marginal or basic modifications, while its junction with older rocks is obscured by Pleistocene sediments. The complex is probably later than pre-Cambrian in age, but in many respects it closely resembles certain well-known pre-Cambrian masses.

The geological description of the norite mass which forms the Colony hills gives little idea of the actual appearance of the country side. The scenery is very picturesque, steep hills and undulating land serrated with valleys, often encircling small bays, are covered with tropical vegetation, while practically everywhere is seen the prevailing red tint of the soil. This soil is characteristic in appearance, it has a rich red colour darkened with humus in the top foot, while "lateritic" concretions generally form from 30 to 70 per cent. of the soil. These concretions vary in size from the microscopic to the most enormous "boulders" many feet, often yards, in extent. The concretions are not very hard and are cut through without much difficulty where cuttings for roads and railways are necessary. Liberally besprinkling the red soils, especially on the hilly slopes, are dark norite boulders, while in some cases the norite mass forming the Colony hills is exposed for large areas. During rainstorms the gutters, streams and rivers run red with the soil brought down from the hills, and it is very easy to see that the soil formed in the hills is being continually washed down to a lower level.

The rainfall in this area varies from 150 in. on the lower slopes (Freetown) of the hills to 180 in. at Hill Station, about 700 ft. up. The average maximum shade temperature is 87° F. and the average minimum shade temperature is 72° F.

Table I.

	First foot	Second foot	Third foot	Fourth foot
Colour ... ..	Brownish red	Red	Yellowish red	Reddish yellow
Stones and gravel (2 mm.)	75.1	77.9	72.4	67.0
Fine earth (2 mm.)	24.9	22.1	27.6	33.0
containing: Coarse sand	29.8	20.3	20.2	15.4
Fine sand	50.2	50.6	50.0	48.4
Silt	1.2	7.2	4.8	7.9
Clay	18.8	21.9	25.0	28.3
Organic matter ... ..	3.0	1.67	1.83	1.69
pH ... ..	4.3	4.3	4.3	4.3
Lime requirement ...	0.20	0.19	0.22	0.24

The soil was described in a previous communication<sup>(1)</sup> and the figures given for typical soils are reproduced in Table I. The sample which was analysed in detail (Table II) was not taken from the same hole as the samples described in Table I but from near the same spot, and although otherwise similar it proved to be somewhat richer in organic matter.

Samples of the various soil fractions were isolated for analysis; they consisted of:

*Large stones.* Concretions greater than 2 mm. in diameter—quartz particles and any small pieces of norite or decomposing norite were avoided.

*Small stones.* From 1 to 2 mm. in diameter. There was no separating out of quartz particles in this and the smaller sized fractions.

*Coarse sand.* From 0.2 to 2.0 mm. in diameter.

*Fine sand.* From 0.02 to 0.2 mm. in diameter.

*Silt.* From 0.02 to 0.002 mm. in diameter.

*Clay.* Particles less than 0.002 mm. in diameter.

These fractions were separated by the ordinary methods of suspension and sieving, and after drying at 100° C. the finer fractions were ground in an agate mortar in preparing the samples for analysis, while the coarser fractions were reduced to powder in a small, end runner, grinding mill. The pulverised samples were fused with fusion mixture until fusion was complete (about 2 hours at the temperature we employed) and the fused mass analysed for silica, alumina, iron, calcium, loss on ignition and organic matter. The difference between the "loss on ignition" and the organic matter (obtained by dry combustion of the sample) was taken as representing "combined water."

For comparison the analyses are also given of the norite rock in the immediate vicinity and the lateritic crust formed on the outer surface of these rocks. In the table given below the "norite rock" sample was taken from the interior of a split rock, while the "laterite crust" was that formed in situ and composing the outside layers of the same piece of rock. In addition an analysis of low grade bauxite found in the near vicinity is added.

The figures in Table II are most interesting. The silica is lowest in the "large stones" or "lateritic" concretions, although here it is shown to be 12.8 per cent. or above that allowed by Fermor's definition of laterite. These concretions, however, which in the main were composed of a smooth soft red material, contained embedded in the red matrix, crystals of gibbsite and a few small quartz grains: these grains became finely comminuted when the sample was ground and could not be

separated from the powdered sample. All the silica found in these concretions is not therefore in the combined state, and it was impossible to estimate exactly the proportion of combined and uncombined silica present. It is possible that both the large and small concretions conform to, or approximate to, Fermor's definition of laterite. The coarse and fine sand fractions can be seen to be composed of tiny concretions, quartz grains and small pieces of decomposing rock, and this accounts for the higher silica content as well as the presence of calcium in the intermediate fractions. The silica is low in the clay fraction and the silica/alumina ratio is 1.10, indicating that nearly 50 per cent. of the aluminium oxide is probably present in a free state.

Table II.

	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	Com- bined water	Organic matter	Silica/ alumina ratio (molec.)
Large stones	12.8	30.0	46.4	Nil	9.6	0.7	0.72
Small stones	15.1	39.0	28.0	1.8	14.8	0.1	0.66
Coarse sand	26.3	34.7	20.0	2.5	12.5	3.9	1.28
Fine sand	29.3	32.1	13.2	2.0	15.2	7.1	1.54
Silt	28.9	29.3	11.2	1.6	13.1	14.2	1.67
Clay	18.7	28.7	9.6	Trace	24.7	15.5	1.10
Norite rock	49.6	17.9	11.9	18.8	0.6	—	4.69
Laterite crust	7.3	41.5	23.2	Trace	20.6	—	0.30
Bauxite (14)	9.5	51.1	14.1	—	25.3	—	0.31

The actual proportion of alumina does not vary much throughout the fractions, rather a surprising feature, but the silica/alumina ratio does. It is smallest in the concretions, biggest in the silt and is again low in the clay.

It will be seen that the silica in the original rock is very high (49.6 per cent.), while the silica/alumina ratio is also high (4.69). The laterite crust formed on the periphery of this rock has a low silica content (7.3 per cent.) and a very low silica/alumina ratio (0.3) comparable—in both cases—with that of the low grade bauxite deposits in the same vicinity.

An examination of the calcium content shows that whereas the large stones contain none, and the clay only a trace (as is also the case with the laterite crust), the sand fractions contain about 2 per cent., while the original rock contains 18.8 per cent.

The iron in the clay fraction is very similar to that in the original rock, but the percentage of iron present increases progressively through the fractions reaching a maximum of 46.4 per cent. in the large stones or concretions, showing that the cementing material present in the concretions is the iron.

*Formation of the soil.* There is little doubt that this soil is being formed *in situ* from the norite rock. The original rock which contains a high proportion of silica and calcium can be seen undergoing laterisation *in situ*, and the decomposing layer (or laterite crust) on the periphery of the rocks can be seen to be a direct continuation of the parent rock, the white crust retaining the structure of the parent rock. The transformation from the hard crystalline rock to the white, soft material, speckled with red, which forms the laterite crust can generally be followed by the naked eye, and is readily seen with a small hand lens. It is as described by Bauer (*loc. cit.*). The change from the rock to the laterite is very sharp and the white speckled material merges into a red coloured laterite crust containing a much higher percentage of iron than that originally present in the rock.

The peripheral decomposition appears to take place only very slowly (if at all) where the rock is exposed to the atmosphere, but takes place more rapidly where the rock is embedded in soil. This is presumably owing to the fact that the period of saturation of the rock is greatest where it is in contact with the soil, and also perhaps the humic acids of the soil may also assist in the decomposition. In any case it is no uncommon thing when moving a large boulder, which is partially exposed to the atmosphere, to find no obvious trace of laterite on the exposed surface, and a layer, half-an-inch to an inch thick, on the buried surface. It is possible, however, that any laterite formed on the exposed surface would be washed away by the next torrential shower of rain.

Laterite then is formed on the surface of the rock, and during the formation of the laterite the more soluble materials, including the calcium, are removed, while much of the silica is also removed. If decomposition only were taking place it would seem that beds of laterite should be formed *in situ*. This actually does happen under some circumstances, and deposits of low grade bauxite (an advanced stage of laterisation) are formed, with the composition shown in Table II; this composition is very similar to that of the laterite crusts but contains rather less iron. In addition to decomposition, however, disintegration takes place concurrently, and the soft laterite encrustations and bits of partly decomposed rock are fairly easily dissociated from the original rocks. This material, where the land is hilly, is gradually transported to a lower level by erosion forming beds of soil, but much often remains *in situ* round the original rocks.

Whether this material, which is composed of lateritised particles and bits of decaying norite with some particles of unchanged quartz,

stays *in situ* or is removed to a lower level, more changes take place. Further decomposition of any undecayed rock goes on while small particles become cemented together by the agency of the iron oxides present, and ever growing concretions enclose small crystals of gibbsite, aggregations of white laterite material, small particles of quartz and possibly bits of only partially decomposed norite, although probably the silica in the latter may be to some extent replaced by the iron during the process of cementation. The result of this cementing action of the iron is to produce in the soil a vast quantity of lateritic concretions of varying sizes, and in some cases this reconstructed "rock" with its characteristic speckled, somewhat spongy, appearance may be seen lying almost cheek by jowl with boulders of the original rock from which it was formed, although in structure and composition there is no point of similarity between the original and final product.

Much of the soil in Sierra Leone appears to be formed in this way, and the stage in which it now exists is shown by the mechanical composition in Table I and the chemical composition in Table II, while the original norite rock from which it appears to be formed is also shown in Table II. There appears to us little doubt that we are justified in calling this soil "laterite," while making the reservation that it will not conform to Fermor's definition of laterite.

*"Lateritic" brown sandy soils.* The sandy soils of Sierra Leone are altogether different in appearance from the red "laterite" soils. They are of a lightish brown colour darkened by humus in the top foot. A geological description of these soils by Dixey<sup>(14)</sup> is given below:

Beds that are believed to be of Pleistocene age are exposed in the cliffs of the Bullom Shore and in the cliffs and stream-beds around Freetown; they extend over the great coastal plain as a thin layer resting upon rocks of much greater age. The deposits consist of varying proportions of sand and clay, and they are generally overlain by a crust of pisolitic laterite. Resting upon these beds are patches of recent sands and silts. Extensive deposits which are also of recent age occur along the coast, especially between the Sierra Leone Peninsula and Liberia; they consist of a succession of sand beaches and silted-up lagoons that now form a low-lying strip of land covered with dense vegetation.

The sample of soil described below is taken from one of these recent deposits near Freetown. In colour the soil is brown and stained with humus in the top foot, in the second and third feet of soil a lighter colour prevails, while in the fourth foot the soil is distinctly yellow. An examination of the soil gave the following results:

Table III. *Brown lateritic sandy soil.*

			First foot	Second foot	Third foot	Fourth foot
Colour	... ..		Brown	Light brown	Yellow	Yellow
Stones and gravel	... ..		0	0.1	0.3	0.5
Fine earth	... ..		100.0	99.9	99.7	99.5
containing: Coarse sand	... ..		29.0	32.2	36.0	29.6
Fine sand	... ..	} per cent.	52.2	44.6	38.6	43.0
Silt	... ..		4.0	6.4	5.3	2.3
Clay	... ..		14.8	16.8	20.1	25.1
Organic matter	... ..		3.3	2.1	1.5	1.5
pH	... ..		4.2	4.2	4.2	4.2
Lime requirement	... ..		0.39	0.31	0.18	0.17

The fractions of this soil were isolated in the same way as in the case of the red "laterite" soil. With this soil, however, the "large stones" were negligible and no analysis was made of the fraction 0.2 mm. and over.

The composition of the fractions is as follows:

Table IV.

	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	Organic matter	Combined water	Silica/alumina ratio (molec.)
Small stones	87.1	6.9	4.0	Trace		1.5*	21.4
Coarse sand	88.6	6.6	2.3	"		0.9*	22.7
Fine sand	75.6	12.4	7.7	"		2.8*	10.3
Silt	42.0	31.1	9.4	0.6	5.9	10.0	2.28
Clay	31.7	31.0	10.8	Trace	5.9	18.2	1.73

\* Equals loss on ignition, organic matter not estimated separately.

It will be seen from the mechanical analysis that there was a large proportion of "sand" present, and the chemical analyses show that the "sand" fractions were composed almost entirely of quartz. In the "fine sand" fraction more particles containing alumina were present and these increased greatly in the silt but could not be separated out quantitatively for analysis.

The clay contained a high proportion of alumina, and as will be seen from the silica/alumina ratio, free alumina is presumably present. It would be most difficult to estimate the combined and uncombined silica present in the whole soil, but in the clay there is at any rate quite enough alumina present for all the silica to be in a combined condition.

A rather curious feature of these analyses is that the iron increases with decrease in the size of the soil fraction. This is absolutely the converse of the condition found in the red "laterite" soil where the large stones contained 46 per cent. of iron oxide (Fe<sub>2</sub>O<sub>3</sub>). The explana-



tion for this lies in the increasing proportion of pure quartz in the larger fractions, there being no concretions cemented together with iron as in the case of the red laterite soil. Calcium was only present as a trace, except in the silt where it reached 0.6 per cent.

This soil, apart from the clay fraction, does not appear to be of a lateritic nature, but it is thought that as the clay is presumably the most important fraction in determining the reaction of a soil, the presence of free alumina in the clay fraction marks off this soil as of a lateritic nature. This point however involves the definition of the term "lateritic" soil and will be discussed at a later stage of the paper.

The deposition of this soil is presumably as described by Dixey (*supra*), and there is no evidence to show the condition of the clay fraction (silica/alumina ratio) when it was deposited. Presumably the silica/alumina ratio was once higher than it now is in this deposit and is slowly decreasing as a result of weathering conditions in the soil. Some evidence of this is shown in a succeeding paragraph, where similar soils from further inland (area of less rainfall) have slightly higher silica/alumina ratios.

In addition to the complete examination of all the fractions in the above examples of our two most important soil types, analyses were made of the clay fractions from other soils from various parts of the Colony and Protectorate.

*Red "laterite" or gravelly soils from the Protectorate of  
Sierra Leone.*

The soils included under this heading are of the same general type as the red soil described above, while a further analysis of a soil clay

Table V. *Description of samples and mechanical analysis.*

(First foot samples unless otherwise described.)

District and geological formation	Stones and gravel	Fine earth	Analysis of fine earth			
			Coarse sand	Fine sand	Silt	Clay
Konno dist.:						
Younger granites	63.5	36.5	37.0	28.0	21.9	13.1
Moiamba dist.:						
Older schists and gneisses	27.5	72.5	23.0	47.6	2.0	27.4
Ditto (fourth foot)	69.6	31.4	15.5	23.5		61.0

from the norite hills of the Colony is also given. The proportion of stones in these soils may vary and the colour may change from a rich red to a reddish yellow; especially is this so with samples taken from a depth,

but in general appearance and characteristics they approximate to the red soils of the norite hills of the Colony.

The analyses of the clay fractions from these samples are given in Table VI.

Table VI.

Analyses of samples described in Table V.

Soil	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	Organic matter	Com- bined water	Silica/ alumina ratio (molec.)
Konno gravel soil	28.6	32.4	6.0	0.6	5.3	25.8	1.49
Moiamba gravel soil	26.8	35.0	7.5	0.1	7.0	21.2	1.30
Ditto (fourth foot)	28.2	36.0	10.3	0.6	3.7	18.3	1.32
Freetown gravel soil	22.8	36.8	14.4	—	27.1		1.05

These figures have two points of interest. The first and fourth foot samples from the Moiamba soil show that the stage of laterisation as evidenced by the soil clay is the same in the fourth as in the first foot. The other point of interest is that the further from the sea (and the less the rainfall), the less the degree of laterisation. This may be merely accidental, and not sufficient figures are yet available to test this point thoroughly. It is, however, highly significant and tallies with the theory that high rainfall is necessary for the formation of laterite.

Table VII describes various samples of sandy soils and grits which have been examined.

Table VII. *Description and mechanical analysis of sandy soils.*

(First foot samples unless otherwise described.)

District and geological formation	Stones and gravel	Fine earth	Analysis of fine earth			
			Coarse sand	Fine sand	Silt	Clay
Colony: Recent	0.4	99.6	55.8	5.4	13.0	25.8
Gbangbama: Recent	—	100	13.0	33.9	31.2	21.9
(swampy conditions)						
Moiamba: Recent	—	100	22.8	66.0	3.4	7.8
Konno grit:						
Younger granites	20.9*	79.1	27.2	20.4	43.4	9.0
Ditto (third foot)	25.0*	75.0	51.4	22.8	19.0	6.8

\* Mostly less than 3 mm.

The analyses of the clay fractions of these soils yielded the results given in Table VIII.

The Konno grit was different both in appearance and chemical analysis from the sands, the "sand" fractions in the grit soils consisting largely of bits of decomposing granite, whereas in the sandy soils these fractions were composed of almost pure quartz.

It will be seen that the soil clays of the sandy soils have a higher

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silica/alumina ratio than the "laterite" gravels, but only in one case does this exceed 2. Another interesting feature is that the further from the coast the higher the silica/alumina ratio; this was found to be the case with the "laterite" gravels, and as it appears to be of some significance. Table IX has been drawn up to indicate the relation between the distance from the coast and the silica/alumina ratio. The rainfall figures as far as they are known are also given.

Table VIII.

Analyses of the clay fractions from the soils in Table VII.

Soil	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	Organic matter	Combined water	Silica/alumina ratio (molec.)
Colony sandy soil	36.2	33.9	8.2	Trace	2.7	17.1	1.81
Gbangbama sandy soil (swampy)	35.7	32.0	7.9	0.2	10.7	13.4	1.89
Moiamba sandy soil	36.6	29.3	4.8	Nil	6.8	19.1	2.11
Konno grit	27.4	28.0	11.8	0.3	41.1	—	1.66
Konno grit (third foot)	26.4	41.9	3.4	0.8	2.2	24.1	1.07

Table IX. *Showing relation between silica/alumina ratio, rainfall and distance from coast.*

Distance from coast in south-westerly direction of meteorological stations or soil analysed	Annual rainfall	Silica/alumina ratio in clay		
		Red gravel soil	Sandy soil	Black grit
0	150	1.05, 1.10	1.73	—
15	—	—	1.81	—
20	—	—	1.89	—
35	112	1.30, 1.32*	—	—
60	105	—	2.11	—
90	99	—	—	—
120	92	—	—	—
150	—	1.49	—	1.66, 1.07†

\* Fourth foot sample.

† Third foot sample.

The "grit" soils are of an entirely different type from either the sand or gravel types and cannot be directly compared with them. Although the evidence is a little meagre it would appear that in Sierra Leone laterisation is proceeding fastest in those regions having the heaviest rainfall.

*Examination of Nicoll's Brook ravine.* Nicoll's Brook is a deep natural cutting near Freetown, which, during the dry season, has only a trickle of water in it, but during the rainy season may have a torrent of some 10 to 15 ft. deep running swiftly through it. The banks are exceedingly steep and the soil and subsoil are exposed to a depth of some 35 ft.

during the dry season. The following is a description of the geology from notes kindly supplied by Major N. R. Junner, of the Gold Coast Geological Department, who visited the cutting.

The formation is probably of Pleistocene age. In the section exposed the surface soil and subsoil down to about 10 to 15 ft. is a mixture of laterite boulders and concretions. Beneath this is a deposit of white very friable sandstone which has become coloured orange-yellow and brown by iron oxides and in places is cemented together by iron oxide forming a compact rock. This sandstone extends down to about 25 to 30 ft. Below the sandstone is a narrow band or platform of limonite and very ferruginous sandstone. This band or plate is about 4 in. thick, extremely hard, and contains in places charred wood fragments. Underlying this plate is a thick bed of tough bluish grey clay containing concretionary nodules of marcasite. The band of limonite is thought to have been formed at an old water level and the iron forming it derived, at any rate in part, from the underlying clay which still contains isolated nodules of marcasite.

Samples of each of these deposits were taken and analysed in the laboratory. The surface soil proved to be normal laterite soil, examples of which are given in Tables I and II; the analysis of this was therefore not considered necessary. Of the other samples the grey clay was examined as a whole without any separation of fractions; the limonite plate was ground in a mill and the pulverised mass examined, it being impossible to separate out any individual quartz grains embedded in the hardened mass. The ferruginous sandstone was likewise ground and the powdered mass analysed, but in the friable sandstone deposit, in which there was hardly any cohesion, the quartz was separated out by sedimentation before the remaining material was examined. A curious point about the fine fraction from this very friable sandstone was that it could not be easily deflocculated, and no matter what flocculating agent was used, most of the fine material flocculated within 2 hours and settled to the bottom of the vessel. By only allowing 2-hour sedimentation, however, it was possible to remove the coarse portion which apparently was almost entirely composed of quartz particles. Only the fine material from this was examined. The results of the analyses are given in Table X.

These figures are interesting in two particulars. They show the similarity in chemical composition of two deposits adjacent to each other yet widely differing in appearance, namely the ferruginous sandstone and the limonite band, and the similarity between the silica/alumina

ratio of the grey clay below the limonite band and the clay separated from the sandstone above the limonite. In both cases the silica/alumina ratio is low and compares with that of the clay fractions separated from the sandy soil (recent deposits) in the Colony (Tables IV and VIII).

Table X.

Description of samples from Nicoll's Brook	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	Com- bined water*	Silica/ alumina ratio
Grey clay	41.4	38.1	0.2	0.9	18.5	1.84
Limonite band	33.4	6.9	51.8	0.3	4.9	8.28
Ferruginous sandstone	29.0	11.0	49.8	Trace	8.4	4.47
Sandstone (fine fraction)	33.2	31.1	19.2	„	14.5	1.81

\* Loss on ignition. There was practically no organic material in these samples.

*The clay fraction of Sierra Leone soils.* The clay fractions of all Sierra Leone soils are remarkable for their high alumina content, and in only one case has the silica/alumina ratio been found greater than 2. The clays from the gravelly soils have the lowest ratios and vary from 1.05 to 1.49, while those from the sandy soils vary from 1.73 to 2.11. The iron oxide varies from 3.4 to 14.4 per cent. and there is usually little, often only a trace, of calcium present; the highest percentage found was in the "grit" soils of Konno where 0.8 per cent. of calcium (CaO) was present.

#### *Classification of soils of a lateritic nature.*

In order to classify soils of a lateritic nature it is first necessary to define the term "laterite" and the adjective "lateritic." While Fermor's definition of a laterite soil is chemical and definite we think that it is inappropriate in that it would not include the red gravel soil of Sierra Leone, where the "stones and gravel," which may form 30 to 70 per cent. of the soil, are concretions which would possibly conform to Fermor's definition of laterite, and the remainder of the soil has a low silica/alumina ratio. This soil, as far as descriptions of laterite soil are concerned, is a typical laterite, and as far as the senior author can judge from visits to the Gold Coast and Nigeria, no more definitely lateritic soils exist in those Colonies than in Sierra Leone. In fact, if Fermor's definition be accepted, we probably have little laterite soil in West Africa. Our red gravel soils have three definite characteristics:

(a) A vivid red colour.

(b) A high proportion of concretionary "stones" with a very low silica content (of which a small proportion is probably quartz).

(c) A low silica/alumina ratio in all fractions, especially in the clay. Such soils have always been known as laterite soils, we think rightly, and it would be a mistake to restrict the term laterite so that it would exclude them. It is thought that for the purpose of soil characterisation the definition should not be based on the chemical composition of the whole soil for the following reasons:

(i) The chemical composition of the highly insoluble (in water) coarse fraction is not so important from an agricultural point of view as the chemical composition of the clay, although on account of its preponderance it would be the deciding factor in determining the proportions of the various constituents present in the whole soil.

(ii) It is difficult to obtain small samples that are really representative of the whole soil; hence all the fractions must be analysed separately.

(iii) In such an analysis particles of pure quartz would certainly be included because of the difficulty of separating them out both in the fine sand and silt fraction where the particles are small, and also in the "stones" where they are cemented into the hard material forming the concretions.

For the above reasons it is thought that an examination of the clay should provide a better basis on which to classify the soil. The clay is generally regarded as the most important fraction of the soil and it is easily isolated and prepared for examination. We have therefore in our work in Sierra Leone regarded the clay as the important fraction.

In considering what should be accepted as lateritic constituents we think that the important one is alumina, and that iron, titanium and manganese, where they occur, should, like quartz, be regarded as unessential constituents. This being so the remaining constituents of the clay are chiefly silica (non-lateritic) and alumina (lateritic) and we suggest that the ratio of silica to alumina, in the clay fraction, should determine whether a soil should be regarded as lateritic or otherwise.

Working on these lines in Sierra Leone, we have examined a number of soils and find that the silica/alumina ratio varies from 1.05 to 2.11. Of these soils, those with a silica/alumina ratio of less than 1.33, we think, should certainly be described as laterite, while those with a ratio of over 1.33 and less than 2.0 should be described as lateritic soils, and those soils with a ratio of over 2.0 should not be described by any term denoting the presence of a high proportion of alumina.

On this basis all our red gravel soils near the coast where the rainfall is over 100 in. a year are laterites, and those of the interior where the

rainfall is less are lateritic soils. With respect to the sandy soils, those near the coast could be described as lateritic, while one sample examined from the interior could not be described by any terminology denoting an excess of aluminium.

It is of course quite arbitrary to make the ratio 1.33 the dividing line between laterite and lateritic soils, and it is realised that the publication of other results of similar analyses may result in shifting, in one direction or the other, the dividing line between what should be called "laterite" and what should be described as "lateritic," but with regard to the upper limit of the "lateritic" ratio, the writers think they are perfectly safe in fixing this at 2.0.

#### SUMMARY.

(1) The formation of laterite and lateritic soils in Sierra Leone has been studied and the mode of formation and the composition of these soils is described.

(2) It is suggested that since the clay fraction is regarded as the most important fraction in determining the reactions of a soil the classification of laterite and lateritic soils should be based on an examination of the clay fraction. It is further suggested that where the silica/alumina ratio in the clay fraction falls below 2.0 the soil should be described as "lateritic," and where this ratio falls below 1.33 the soil should be described as laterite.

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Fig. 1. Cutting through mass of Norite rock showing the formation of laterite between the rocks along the planes of cleavage. Signal Hill, Freetown, Sierra Leone.

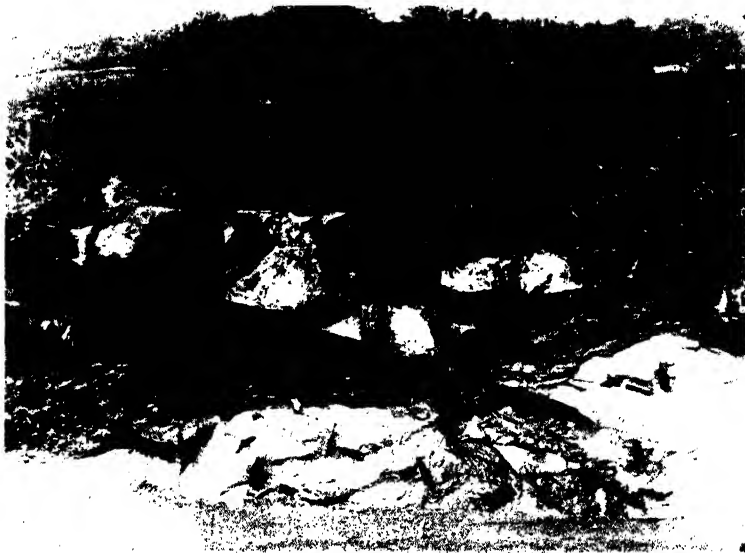


Fig. 2. Cutting through Norite rock showing the formation of laterite between rock boulders. Note the typical layers of laterite round the Norite boulders. Hill Station, Freetown, Sierra Leone.







Fig. 3. Cutting through laterite soil showing lateritic concretions. The crop growing above is maize. Congo Town, Freetown, Sierra Leone.



Fig. 4. Hill slope showing outcrop of Norite rock (just below bungalow) with red laterite soil in foreground. The "Bush" has been cleared from the bungalow area by the Sanitary authorities. Hill Station, Sierra Leone.



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# STUDIES IN CROP VARIATION.

## IV. THE EXPERIMENTAL DETERMINATION OF THE VALUE OF TOP DRESSINGS WITH CEREALS.

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(With One Text-figure.)

### I. INTRODUCTION.

THE practice of applying nitrogenous fertilisers to cereal crops as a top dressing is one which has become firmly incorporated into normal farm routine.

The classic wheat experiment on Broadbalk field shows that to apply all the nitrogenous fertiliser at the time of drilling the seed in autumn leads to a diminution in crop when compared with the yield of a plot in which only a quarter of the nitrogenous fertiliser was applied in autumn.

Over the 49-year period 1878–1926, during which time the treatments of the plots have been uniform, the average yields are as follows:

Plot No.	Autumn dressing lb. of N.	Spring dressing lb. of N.	Average total grain in lb. per acre	Differences
15	86.0	—	1647	
7	21.5	64.5	1894	247
6	21.5	21.5	1313	581

The loss of crop due to corresponding loss in nitrogen leached out of the soil by winter rains is serious in spite of the fact that the 86 lb. of nitrogen applied gives a substantial margin over the quantity required by the plant to support a 1900 lb. crop (about 50 lb.). Comparison with plot 6 shows that the added single dressing of 43 lb. nitrogen on plot 7 gives an increase of 581 lb. of grain in this region of crop yield, so that the loss in yield due to autumn application of 64½ lb. of nitrogen amounts to approximately 16 lb. of nitrogen (28 per cent.) lost before the time of spring dressing. The 40 lb. per acre of nitrogen lost annually during the early years of the drainage experiment from the uncropped 1/1000 acre drain gauge suggest that the gross loss is possibly much heavier. Whilst therefore a spring application of nitrogen in some form is undoubtedly the most beneficial, there remains the important point as to whether the time of that application can be determined within

reasonable limits in order to ensure the production of something approaching a maximum crop. The following field experiment was designed to investigate this point, and the detailed description which follows is published not as a solution of the problem but as showing what is hoped will prove a fruitful method of tackling such a question. An attempt has been made to view the problem as a whole and to put the investigation on a broad basis. The design of the experiment, instead of being only a matter of secondary importance, as has often been the case in field experiments, is in this instance paramount and has been determined by a detailed consideration of the nature of the problem, the accuracy deemed necessary, and the practical difficulties which had either to be allowed for or overcome. Such a contribution in the sphere of field experimental technique will, it is considered, be better presented in the form of a description of the experiment as a step in a specific experimental programme rather than as an abstract example of method.

## II. PLAN OF EXPERIMENT.

### 1. *General.*

The site of the experiment was one of the typical "clay loam with flint" fields common in the district, and at the time was considered to be deficient in organic matter. The experimental crop was oats (Grey Winter) which marked the end of a long rotation of corn broken only once with a clover ley. The previous history of the field is shown below in tabular form.

Year	Crop	Type of manuring
1920-1	Potatoes	Dung and artificials
1921-2	Wheat	None
1922-3	Barley undersown	"
1923-4	Clover	Basic slag and potash
1924-5	Wheat	Complete artificials

The seeding took place under favourable conditions and germination was fairly rapid. The crop was tillering before the winter set in and stood the winter well, showing a strong plant when the plot site was chosen on February 20, 1926. The whole field had been dressed with a mixture of artificial manures, of which the composition per acre was: sulphate of ammonia,  $\frac{1}{2}$  cwt.; superphosphate, 2 cwt.; muriate of potash, 1 cwt. Throughout the period of growth the plant suffered no check, but wind and rain in mid-July laid the crop badly. By cutting before the crop was too ripe the plots were cleanly harvested, and this operation probably did not contribute to the experimental error more than is

normally the case. The order of lodging of the plots was on the whole definitely marked, the later and the heavy dressings of nitrogen starting first; those not receiving nitrogen were clearly less damaged.

The dates of operations and stages of development were as follows:

Operation or stage of development	Date
Sowing ... ..	October 9 and 10
Rows visible ... ..	November 15
50 % tillering (early dressing applied)...	March 15 and 16
Maximum shoots (late dressing applied)	June 5
Cut ... ..	July 28–August 7
Carted ... ..	August 17 and 18
Threshed ... ..	January 1927

## 2. *Experimental scheme.*

In planning this experiment, allowance had to be made for the fact that it was proposed to carry out similar trials in subsequent years, and consequently some convention with regard to the two dates of top dressing (designated for convenience “Early” and “Late”) had to be arranged. A calendar basis would obviously be unsatisfactory, but if definite stages in the life cycle of the crop could be used, a measure of comparability could be assured. The two stages chosen were firstly the recommencement of growth in the spring as shown by the production of tillers, and secondly the time of maximum tiller development. The first stage ensures that the nitrogenous fertiliser can actually be used by the plant, and is not lost as leached nitrate at a time when the soil temperature is too low for plant growth; the second coincides within limits with the beginning of rapid development of the grain. Periodical counts of the number of shoots per metre row were made throughout the season on the basis of three random rows per plot. For the purpose of ascertaining maximum shoot number these were frequent but not extensive.

Attention was not confined either to one moderate application or to one form of nitrogen, there being applications 1 and 2 cwt. per acre of sulphate of ammonia and dressings equivalent in nitrogen (94.5 lb. and 189 lb.) of muriate of ammonia.

By confining the experiment to one quantity and to one kind of nitrogen only, a greater number of replicates could have been afforded in the space and with the labour available, but the circumstances would have been too special to have had much value, and nothing would have been known regarding the possible differential behaviour with respect to time of application of the two quantities of the two types of nitrogen.

Moreover, it is one of the advantages of the experimental method to

be described that in carrying out such a comprehensive or survey experiment not only is extra information obtainable but the reduction in pure replications which it involves does not (within definable limits) reduce the accuracy of the experiment. This follows from the fact that in a systematically designed experiment every plot is of use in making every possible comparison, and the whole number of plots is available

	2 M EARLY	2 S LATE		2 S LATE			1 S EARLY
1 S EARLY	1 M EARLY	1 M LATE	1 S LATE	2 M EARLY	2 M LATE	1 M EARLY	1 M LATE
	2 M LATE		2 S EARLY		1 S LATE		2 S EARLY
2 S EARLY	2 M EARLY		1 M LATE		2 S EARLY	2 S LATE	2 M LATE
	1 S LATE	1 S EARLY	1 M EARLY	1 M LATE			1 S LATE
2 M LATE		2 S LATE		2 M EARLY		1 M EARLY	1 S EARLY
2 S EARLY	2 M LATE	1 S EARLY	2 M EARLY	2 S LATE	2 S EARLY	2 M EARLY	
		1 M LATE		1 M EARLY	2 M LATE		1 M LATE
2 S LATE	1 M EARLY		1 S LATE			1 S EARLY	1 S LATE
2 M EARLY	1 M EARLY	2 M LATE	2 S LATE	1 S EARLY			1 S LATE
1 S LATE			1 M LATE	1 M EARLY	2 S EARLY	2 M LATE	
1 S EARLY		2 S EARLY			2 M EARLY	2 S LATE	1 M LATE

Fig. 1. A complex experiment with winter oats. (Reproduced from the *Journal of the Ministry of Agriculture* by permission of the Controller of H.M. Stationery Office.)

for the estimation of the error applicable to any group of them. An amplification of this and subsequent points is given in the section devoted to the analysis of the data and method.

One of the largest contributory causes of error in field trials is the unavoidable heterogeneity of the soil. By special arrangement much of this disturbing element can be eliminated from the estimates both of the average yields and of the errors to which they are subject. In the



particular case under consideration disturbance was further added to by the fact that it was found necessary to superimpose the 1/40 acre plots of which the experiment consisted on a series of 1/15 acre plots in the clover shift which had been heavily dressed with varying quantities of markedly different basic slags. For this reason instead of a fourfold replication an eightfold one was used. The results are thus a unique test of the success of the method in eliminating any heterogeneity of this kind.

Fig. 1 shows in diagrammatic form the lay-out of the experiment. The dimensions are not to scale. The following points are noteworthy and form the essence of the lay-out.

1. The 96 plots are grouped into eight blocks resembling window panes and within each block each treatment occurs once. The fact that there are four no-nitrogen plots in each block is due to a special reason.

2. Within each block the arrangement is arrived at by the process of drawing lots.

3. The dimensions of the blocks are arranged so as to make them as compact as possible. The correlation between fertility of adjacent sections of a field is high, and in this way the comparability of plots within a block is maintained at as high a level as possible. In this case the dimensions were  $175 \times 197$  links, including paths (5 links) passing between all adjacent plots.

It has been generally recognised that the introduction of replication into agricultural experiments has made possible a greatly increased accuracy, and that such increased accuracy was urgently required if the results of experimental trials were to carry any weight in guiding farm practice. It has been realised much more slowly how very great is the increased precision required for the solution of practical problems; this conclusion has been forced upon workers both in variety and manurial trials by a realisation that the advantages of improved farming, while enormous in their effects upon the general revenue of the agricultural community, and large in relation to the economic return for the individual farmer's skill, are yet small when expressed as a percentage of the gross yield of the crop. In applying a manure the farmer is concerned not merely with the increased selling value of his crop, which may easily be brought up by 10 or 20 per cent., but with the balance left after the increased cost of growing it has been allowed for; and in this balance an advantage or loss of 5 per cent. represents a very considerable item. It is probable, therefore, that the advantages of direct experimentation in the field will not be fully open to exploitation for the

benefit of agriculture, until it becomes possible to carry out field experiments with confidence that the standard error of the values obtained will not much exceed 1 per cent.

The main advance in recent agronomic progress has been due to increased replication; nevertheless it is evident that many replicated experiments have yielded very disappointing results. This is partly due to an insufficient realisation of the extent to which replication may with advantage be practised, but in large part also to the fact that the satisfactory design of replicated experiments involves other considerations, practical and theoretical, to which adequate attention has very seldom been paid. These may be considered under three heads: (i) the geometrical arrangements of the plots in the field, so as to minimise the errors due to soil heterogeneity, (ii) the systematic choice of the combinations of treatments to be studied, so that every plot yield may be available to throw light upon every question which it is desired to answer, (iii) the provision by the field arrangement of a valid system for the estimation of the standard error of the results, or of the precision to be attached to them. These considerations may be illustrated by the discussion of the top-dressing experiment under consideration; in this discussion we must seek for the reasons, in so far as such reasons can be assigned *a priori*, for the exceptionally high precision actually attained.

To obtain the maximum precision in the comparison to be made between sulphate and muriate it is important that corresponding to every plot receiving sulphate there shall be a plot, similarly treated in respect of quantity and time of application, in which the sulphate is replaced by muriate. Similar considerations apply to the comparison of early with late application, and to the comparison of single with double quantity. These requirements are satisfied by making up the manurial treatments in all possible combinations, a system which has the additional advantage that any interaction which may exist between the effects of the three different factors upon yield may also be detected with the same precision. Thus, in testing whether the differential effect due to the second dose is greater with the sulphate than with the muriate, or whether it is greater in the early than in the late application, our comparisons will be based upon the means of 32 plots, corresponding each to each in other particulars. The remaining two-factor action, representing the comparison between sulphate and muriate in the advantage, if any, of early dressing, and the three-factor action, representing the comparison of this effect in the single and the double quantities, may be made likewise with a precision appropriate to the

comparison of the means of 32 plots. In order that the same accuracy may apply to the comparison between the plots receiving none, with the plots receiving one and two doses, the former must also be given 32 plots, making a total of 96. Every plot is therefore made to add to the precision of the conclusions on every point examined; regarded as an experiment of 12 treatments in eightfold replication, comparisons should be available on all different questions, but since there is no agricultural distinction to be drawn between the sets of 4 plots receiving no top dressing, the number of agriculturally relevant questions to be answered is reduced to 8; it is an important point in the system of combinations employed that each of these 8 questions will be answered with a precision appropriate to the means of 32 plots. The experiment thus gains in efficiency not merely from its size, but also from its complexity.

A further advantage of combining several factors in the same experiment lies in the circumstance that the action of each factor is examined not merely in one particular set of circumstances, but in four different sets. The conclusions to be drawn as to the action of each factor are thus given a wider inductive basis than would be the case with a single factor experiment of equal precision. This advantage may be summarised by saying that an experiment of this type combines the advantages of wide exploration with those of precision.

The geometrical arrangement of the plots with a view to the elimination of as much as possible of the variance in fertility of the area used, is a most important consideration, to which many writers have paid attention. The problem of the reduction of error by skilful arrangement is found, however, to be intimately involved in the problem of obtaining from the results a valid estimate of the errors remaining. The statistical procedure known as the analysis of variance brings to light the fact that of different experiments represented by assigning the same physical plots of land differently to the chosen treatments, and using the same statistical procedure for the estimation of error, those experiments which are in reality the most accurate will appear to be least so, and *vice versa*. Because each element of field heterogeneity must produce its effect either in the real errors, or in the differences between replicates upon which the estimate of error is based. The experimenter, therefore, who wishes to decrease his real errors, and at the same time obtain a valid estimate of them, will be obliged to make his methods of statistical estimation conform to the field situation, a precaution obviously necessary on theoretical grounds, which has been very greatly overlooked. In practice we can in fact eliminate in the field the important elements

of soil heterogeneity represented by the differences between whole blocks of plots, by the simple method of putting one replicate of each treatment into each block. The differences between the blocks as wholes is thus completely eliminated from the comparisons between treatments, and a parallel elimination can be made (Fisher, 1925, p. 224) in the estimation of error. The errors which remain to disturb our comparisons are those due to fertility differences between plots of the same block, and in order that these may be estimated with validity the only adequate precaution is to arrange the different treatments in the same block wholly at random.

The experiment, then, is of the general type known as "randomised blocks," there being in this case eight blocks, each containing 12 plots, the treatments of which are assigned by pure chance. The coherence between field arrangement and laboratory calculations, to the necessity of which attention has been called above, may be followed in the next section.

### III. YIELDS AND ANALYSIS.

For calculations the yields of grain and straw are best set out as in the following table, wherein to avoid superfluous decimal places the grain yields are given in eighths and the straw yields in halves of a pound, these being the units of weighing.

The marginal totals show at once that, while there are considerable differences in the fertility of the eight blocks of land used, the difference between the treatments is very small, especially in the yield of grain, of an order which could only be detected in a very precise experiment. The arithmetical analysis will be designed to separate the components of the observed variation due to the different blocks of land, due to each item in the differences in the treatments, and due to the residual errors by which the results obtained are effected.

A simple preliminary, which serves both to guide and to interpret the calculations based on the yields, is to partition off the total number of degrees of freedom available into parts representing the different causes of variation, the effects of which can be separately evaluated. The total of 96 plots will yield 95 independent comparisons, and these 95 degrees of freedom would have been partitioned, had 12 different treatments been used, into

7 differences between different blocks of land,
11 differences between different treatments,
77 differential responses to treatments in different blocks.
<hr/> 95

Owing to the identity of the treatment of the four plots receiving no top dressing in each block, we have in fact only eight different treatments, and only 56 degrees of freedom representing differential response; the remaining 24 degrees of freedom represent the differences among the undressed plots on each block, each block yielding three such comparisons. The partition required is therefore

7 differences between different blocks of land,

8 differences between different treatments,

24 differences between plots similarly treated in the same block,

56 differential responses to treatments in different blocks.

95

Table I. *Grain.*

Block treatment	I	II	III	IV	V	VI	VII	VIII	Total
0	491	634	604	732	629	677	551	650	4968
0	524	668	599	690	632	676	636	644	5069
0	545	666	502	710	671	703	506	717	5020
0	577	679	689	660	622	637	670	678	5212
1 <i>SE</i>	620	646	681	644	706	615	552	726	5190
1 <i>SL</i>	644	745	542	711	705	637	543	646	5173
1 <i>ME</i>	523	713	686	688	692	612	635	748	5297
1 <i>ML</i>	601	693	685	714	099	697	701	746	5536
2 <i>SE</i>	664	693	666	516	656	663	657	683	5198
2 <i>SL</i>	514	637	697	710	633	595	697	712	5195
2 <i>ME</i>	550	708	663	673	671	626	655	671	5217
2 <i>ML</i>	521	661	594	730	625	644	745	747	5267
Total	6774	8143	7608	8178	7941	7782	7548	8368	62342

Table II. *Straw.*

Block treatment	I	II	III	IV	V	VI	VII	VIII	Total
0	166	244	204	299	220	288	201	252	1874
0	192	281	208	288	231	291	226	257	1974
0	187	243	198	310	267	243	181	314	1943
0	197	333	276	279	226	273	281	244	2109
1 <i>SE</i>	242	321	261	317	255	381	216	295	2238
1 <i>SL</i>	267	382	201	316	280	285	200	309	2240
1 <i>ME</i>	215	330	298	381	300	294	256	284	2358
1 <i>ML</i>	212	292	265	255	238	309	283	324	2178
2 <i>SE</i>	322	370	284	323	232	393	351	363	2638
2 <i>SL</i>	200	261	259	361	234	258	306	376	2255
2 <i>ME</i>	260	318	286	340	362	400	276	385	2607
2 <i>ML</i>	203	275	207	331	229	266	276	328	2115
Total	2663	3650	2927	3800	3074	3631	3053	3731	26529

The variation in yield both of grain and straw will be analysed into corresponding portions by a similar partition of the sums of squares of the deviations from the general mean. Equally the co-variation of grain with straw may be analysed by partitioning the sums of the products

of the deviations. The large groups of 24 and 56 degrees of freedom represent residual errors not eliminated by the method of experimentation, and therefore provide estimates of corresponding weight of the errors to which our results are subject. The 24 refer only to the error variation of the undressed plots, while the 56 are principally affected by the plots receiving some kind of top-dressing.

The sum of the squares of the deviations of the block totals from their mean provides a measure of the differences between blocks; this will be divided by 12, the number of plots in the block, and by a further factor to reduce to a basis of pounds per plot, namely 64 for grain, 4 for straw, and 16 for the product. The results are shown as the first line of Table IV.

The eight degrees of freedom representing manurial response may, with advantage, be treated individually; thus, taking for example the contrast of Late versus Early application, we find the 32 plots dressed late yielded 269 more units of grain and 1053 units less of straw than the 32 plots dressed early. The squares and products of these differences divided by 64, the number of plots in the comparison, and by the further factors necessary to reduce to pounds of produce, give the corresponding contribution of the one degree of freedom for "time of application" to the total of the eight degrees for manurial treatments in Table IV. In dealing with quantity the same method may be employed for the contrast of double versus single dressings, but if so the remaining contrast of no dressing versus the mean of the single and double dressings requires a divisor of 48 instead of 64, since on taking the mean we have somewhat diminished the sampling errors. The sum of these two contributors may be equally and symmetrically obtained from the deviations of the three totals each of 32 plots from the mean of the three, by squaring, adding and dividing by 32. The totals thus obtained for the eight manurial degrees of freedom are shown in Table IV; it will be useful in addition to show the actual differences of these eight comparisons separately with their proper signs (Table III).

Table III.

	Grain	Straw
Late <i>minus</i> early ... ..	+ 269	- 1053
Muriate <i>minus</i> sulphate ... ..	+ 561	- 113
Single <i>minus</i> double ... ..	+ 319	- 601
<i>ML + SE - ME - SL</i> ... ..	+ 309	- 291
<i>M 2 + S 1 - M 1 - S 2</i> ... ..	+ 379	- 229
<i>E 2 + L 1 - E 1 - L 2</i> ... ..	+ 175	+ 697
<i>ML 1 + ME 2 + SL 2 + SE 1 - SL 1 - SE 2 - ML 2 - ME 1</i>	+ 203	- 73
$\frac{1}{2}$ (single + double) <i>minus</i> undressed ... ..	+ 767.5	+ 1414.5

It should be observed in passing that with the exception of the large effect ascribable to the first dressing, and to the interaction of quantity and time of application the effect upon the grain is in the six other cases of opposite sign to the effect upon the straw.

The analysis of variance in Table IV is completed by calculating the amounts ascribed to the 24 degrees of freedom representing the variation of the undressed plots in the same block. The squares and products of the deviations of each of these plots from the mean of the four are therefore added, and reduced to pounds per plots. The amounts ascribable to the remaining 56 degrees of freedom are then immediately obtainable by subtraction.

For each of the four causes of variation into which the total has been divided a correlation between grain and straw may at once be calculated simply by dividing the sum of the products by the geometric mean of the two sums of squares. These correlations are shown in the last column of Table IV. The values found for the blocks and for the treatments being based on only a few degrees of freedom, are of course liable to large sampling errors, and cannot in fact be taken as significantly different from the remaining two better established correlations. If, however, we confine attention to the eight treatments which received single or double dressings, the correlation for the seven comparisons among them is  $-0.468$ , which is now significantly different from all of the others. This fact will have an important bearing in the interpretation of the results and serves to illustrate a principle of some importance in the use of the comparatively precise types of experiments now possible.

In order to test the significance of each apparent effect, the sums of squares and products of Table IV are each divided by the corresponding degrees of freedom; this shows at once which effects have a larger share of the total variation than that assigned to random causes, and thus forms a basis for testing their significance; the mean squares and products are shown in Table V.

Table IV.

	Degrees of freedom	Sum of squares		Sum of products	Correlation
		Grain	Straw		
Blocks	7	2,286.4	27,556.8	+ 6,793.7	+ 0.8559
Treatments	8	387.0	18,667.1	+ 989.4	+ 0.3681
Errors	24	773.2	5,491.2	+ 1,526.7	+ 0.7409
	56	2,508.8	18,556.3	+ 3,723.4	+ 0.5457
Total	95	5,955.4	70,271.4	+ 13,033.2	+ 0.6371

Table V.

	Degrees of freedom	Mean square		Mean product
		Grain	Straw	
Blocks	7	326.6	3,936.7	970.5
Treatments	8	48.4	2,333.4	123.7
Errors	{24	32.22	288.0	63.61
	{56	44.80	331.4	66.49
	80	41.02	300.6	65.63

The two independent estimates of error do not differ appreciably in the case of straw, so that the values of the variance derived from 80 degrees of freedom may with confidence be adopted; this is 300.6 and its square root 17.84 may be taken as the standard error in pounds of straw for a single plot. The standard error for the total of 32 plots is thus 98.1 lb., or 2.218 per cent.; while that for the difference between two such totals is 138.7 lb. or 277 units of half a pound. Reference to Table III shows that the advantage of early over late dressings, the advantage of double over single dressing, and the differential advantage of applying the double dressing early are all significant, in addition, of course, to the advantage of the dressed over the undressed plots.

In the case of grain the two estimates of error show an interesting apparent discrepancy which may be used to illustrate the method of testing doubtfully significant effects. From the two values of the mean square is calculated a quantity,  $z$ , defined as half the difference of their natural logarithms, we have

$\log_e$	4.480	1.4996
$\log_e$	3.222	1.1700
Difference		<u>0.3296</u>
$z$		0.1648

The question whether so large a value of  $z$  will often occur by chance, when comparing a value derived from 56 with one derived from 24 degrees of freedom may be resolved at once from the table of the 5 per cent. points of the  $z$  distribution (Fisher, 1925, p. 210) which shows that in this case the 5 per cent. point lies between 0.2749 and 0.3425. The discrepancy is therefore quite insignificant, and as in the case of straw, the value derived from the whole 80 degrees of freedom may be used with confidence.

This value 41.02 shows that the effects of the different blocks of land used is very distinct, but that little effect upon grain yield has been produced by the different manurial treatments. The standard error in grain yield of a single plot is only 6.405 lb., and that of the



difference between two totals of 32 plots is therefore 51.24 lb. or 410 units of 2 oz. each. Of the seven comparisons between treated plots only one, that of muriate versus sulphate exceeds this value, and that to an insufficient extent to be judged significant. The comparison of dressed with undressed plots has a standard error of 355 units, and the observed difference of 767.5 is therefore significant.

The significant response in the case of grain is therefore confined to the quantity of nitrogenous top-dressing, the effects of kind and date of application being inappreciable even with the low standard error attained. The quantitative effects may be summarised as in Table VI.

Table VI.

	Quantity of top dressing			Standard error
	None	Single	Double	
Grain, bushels per acre	75.4	78.9	77.7	1.08
„ per cent.	97.5	102.0	100.5	1.39
Straw, cwt. per acre	44.1	50.3	53.7	1.10
„ per cent.	89.3	101.9	108.7	2.22

The figures for straw show in addition certain differential responses involving quantity and time of application, but no effects in the four comparisons involving sulphate versus muriate; the significant effects are therefore summarised in Table VII.

Table VII.

	Single early	Double early	Single late	Double late
Straw, cwt. per acre	51.3	58.6	49.3	48.8
„ per cent.	104.0	118.6	99.9	98.9

The advantage shown by the double dressing is thus confined to the early application; at this time the second dose yields 14.6 per cent. increase, nearly as much as the increase of 14.7 per cent. ascribable to the first dose. In the late application either the single or the double dressing may be said to give an increase of about 10 per cent.

#### IV. DISCUSSION.

Since in the design of the experiment care was taken through the random arrangement of the plots within blocks, to ensure that the estimate of error should afford a reliable measure of the actual errors to which the comparisons made are subject, the extremely low standard errors obtained in Table VI call for some explanations. This is the more important since all experimenters will desire to obtain a similar

accuracy in tests involving questions of practical profit or loss. The main factors in the success of the present trial are clearly three: (i) the adequate use of replication, namely in eightfold in place of the triplicates and quadruplicates usually employed, (ii) the choice of combinations of treatments in such a way that each plot yield recorded contributes to the accuracy of all the comparisons desired, (iii) the elimination of the effects of soil heterogeneity by the method of arrangement adopted in the field. It is worth while to emphasise that only when weight is given to all three of these considerations can high accuracy be expected from field trials.

It is to be noted that when attention is given to the above points the conclusions from the results are drawn without any complex statistical analysis by simple arithmetical additions and subtractions. The only tests of significance required are those of which the theory is now fully understood, and for which adequate tables are available.

The yields of grain and straw obtained from all plots are remarkably heavy. Nevertheless, the early nitrogenous dressings produced a considerable increase of about 7.3 cwt. of straw to the acre for each cwt. of sulphate of ammonia or its equivalent in muriate. A large part of this increase was undoubtedly due to increased tiller formation, and in such a heavy crop it would be surprising if it should be reflected proportionately in an increased yield of grain. Indeed the additional yield in grain from either the single or the double dressings is only about 3 bushels to the acre. We may conclude that much of the additional tiller formation was superfluous in the sense of not leading to additional ears. With the late dressings in June the condition limiting the effect of the top dressing had become much more stringent; no additional yield of straw was produced by the second cwt. of manure, and no additional grain by either the single or the double dressing. This again fits with the view that such late dressed nitrogen as was taken up by the plant, was utilised only in additional and abortive tillers.

The correlation between grain and straw found in Table IV indicates that in the differences between different treatments, though not in the larger differences between blocks of land, some factors were at work acting antagonistically as between grain and straw. Thus the differences in grain yield on the treated plots, though individually insignificant, yet in relation to the straw yields of the same treatments show a significantly subnormal correlation. The same conclusion is suggested, though not so clearly demonstrated, by the low grain-straw correlation in the differential responses of the different treatments on the different blocks

of land. The values of Table III show that this effect is not confined to any one manurial contrast but emerges in considering the aggregate of all. It is not improbable that we have here an indirect effect of the laying of the crop, which has interfered in other ways remarkably little with the success of the experiment, but which may well have hindered grain maturation somewhat more on plots having the greatest abundance of straw. If this is the case the straw yields will afford the better basis for estimating the effects of the manurial treatments on plant nutrition, while the laying of the crop and the small response in additional grain, represent a sporadic risk of loss to which heavy crops are particularly exposed.

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# ON THE ALGAE OF SOME NORMAL ENGLISH SOILS

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(With One Text-figure.)

## I. INTRODUCTION.

In two earlier papers (Bristol, 1919 *a* and 1920 *a*) the writer gave an account of the algae which appeared when small portions of carefully dried samples of English soils were placed in sterile culture vessels with a sterile solution of mineral salts. The samples of soil investigated had all been air-dried for at least a month before examination, and some had been stored in a dry condition for longer periods up to as much as seventy years. All of the samples yielded algae, and sixty-eight species were described. The object of the preliminary desiccation of the soil samples was to ensure that only those species would appear in the cultures which were capable of forming resting spores under conditions of gradually increasing drought such as occur in the field under natural conditions, and might therefore be presumed to be regular inhabitants of this somewhat inhospitable habitat. The frequency with which certain species appeared from the different soil samples led to the conclusion that there is in this country a widely distributed ecological plant formation in cultivated soils consisting of moss protonema and algae, of which the most important species are *Hantzschia amphioxys* (Ehr.) Grun., *Trochiscia aspera* (Reinsch) Hansg., *Chlorococcum humicola* (Naeg.) Rabenh., *Bumilleria exilis* Klebs and to a less degree *Ulothrix subtilis* Kuetz. var. *variabilis* (Kuetz.) Kirchn.; further, that other species of typical soil algae occurring somewhat less frequently give rise to smaller plant associations within this formation. Attention was called (Bristol, 1920 *a*, p. 48) to the fact that certain species of algae which are regarded as typical and commonly occurring soil forms either were completely absent from the cultures or occurred only rarely, and it was suggested that the drying of the soil samples might possibly be responsible for their absence.

Recently Moore and Carter (1926), in describing the algae which they have obtained from a number of freshly gathered subterranean samples of soil from the Missouri Botanical Garden, have drawn attention to the frequent presence of some of these species in their American

cultures and have suggested somewhat hastily that their omission from my earlier English lists was possibly due to their being overlooked or wrongly identified. There is no denying the possibility of error, owing to the extreme difficulty of distinguishing between the unicellular forms in a mixed culture, but data have accumulated during the past seven years which show that at least three other factors contribute towards these omissions besides those suggested above. Foremost among these is the degree of desiccation to which my samples were reduced before culture; this factor has been entirely ignored by Moore and Carter although it was sufficiently emphasised in the original paper. The second factor of importance is that of competition between the various species present in mixed cultures, and the third a perhaps excessive caution which deterred me from including in the lists any forms which could not be identified with certainty at that time. Three-quarters of the cultures contained nine or more species of algae in a comparatively small volume of liquid, and on the first examination a number of unicellular or developmental stages were drawn (including, as it happens, some of the species under discussion), of which the subsequent history could not be determined with certainty, owing to the fact that other species grew so much more rapidly as completely to obscure them or even to kill them. Inability to verify or amplify the original incomplete observations was therefore regarded as a sufficient reason for omitting all reference to the organism concerned.

The criticisms of Moore and Carter have, however, drawn attention to the fact that up to the present there is no published account of the algal flora of a freshly gathered English arable soil; the object of the present paper, therefore, is to remedy this omission by placing on record a number of observations that have gradually been made during the past seven years, and which throw some light on the conditions under which the algae live in the soil.

## II. EXPERIMENTAL METHOD.

Most of the soil samples investigated have been taken from certain of the historic experimental plots at the Rothamsted Experimental Station, and have been examined by means of cultures in mineral salts solutions, set up with different degrees of dilution of the well shaken soil suspension immediately after collection of the sample.

It was hoped by these means to devise a method for determining the numbers of individuals per gram of soil which would enable the sequence of changes undergone by the different species in the soil to

be followed systematically. Success has unfortunately been only partly achieved, for while the method finally adopted is useful in giving a minimum estimate of the numbers of green algae and diatoms present, it is entirely worthless in so far as the blue-green algae are concerned, because mere mechanical shaking is insufficient to break up the tough mucous sheaths with which these organisms are surrounded. A striking proof of this fact was afforded by a surface sample of soil collected in July 1920 upon which there was visible to the naked eye a continuous stratum of blue-green algae about a millimetre in thickness; the number of blue-green individuals present, according to the dilution cultures, was not more than 30 per gram of soil, though the same series of cultures gave a total of nearly 62,000 green algae and diatoms, which were much less evident in the sample. Such quantitative results as have been obtained apply therefore solely to the green algae and diatoms: they must, however, be regarded as minimum figures for the green algae, because there is as yet no available information in regard to the degree to which these algae are separated on shaking; and though doubtless many of the "individuals" are single cells, it is extremely probable that others may consist of clumps of 2, 4, 8 or even more cells. The dilution method finally adopted has been adapted from one in use in the General Microbiology Department of the Rothamsted Experimental Station. Full experimental details and statistical tables<sup>1</sup> have already been given elsewhere by the writer (Bristol Roach, 1927), and it suffices here to say that the method depends on setting up triplicate cultures containing 5 c.c. of each of a series of 13 half-and-half dilutions of a well shaken soil suspension of known strength. After a period of growth the cultures are examined microscopically and records made of the species present in each. By noting the number of cultures from which any given species is absent it is possible to estimate statistically the number of individuals of the species per gram of soil that would be likely to produce such a result, when random sampling is the only factor to be considered.

The test of the reliability of the results is that the first record of absence and the last record of presence of any species must not be further apart than seven successive dilutions in the series: a separation greater than this suggests the working of some factor other than random sampling, such as competition or the toxic or feeding effect of some other organism, and no reliance can then be placed on the result. In practice, it has been found that for the diatoms and for most of the green algae

<sup>1</sup> Calculated from one constructed for the General Microbiology Department by Dr R. A. Fisher.

the results lie well within the prescribed limits, but there are certain species, notably *Heterococcus viridis* Chod. and *Chlorella* sp., on which the effect of competition is very marked. This is shown by the fact that these species frequently may be recorded quite regularly in the higher dilutions of a series and yet be absent entirely or recorded with a query from some of the stronger suspensions where their occurrence would normally be expected as a matter of course. Moore and Carter themselves give a hint of this important factor, without apparently realising the full significance of their observation, for in their account of the alga identified by them as *Botrydiopsis arrhiza* Borzi they say that it is rarely present in great abundance but that "when there is not too much competition with other species it multiplies rapidly and may form an abundant growth." In estimating the probable numbers of individuals in a given sample of soil it is possible that negative records in certain of the heavier suspensions should therefore be ignored when such records are followed by a regular succession of positive records for the higher dilutions of the series.

The factor representing the probable experimental error of the method, due to random sampling, is about 2 to  $2\frac{1}{2}$ , so that one number must be more than twice as great as another to be significantly different from it. On two separate occasions the method was tested by examining a sample of soil consisting of several separate 1-6 inch borings carefully mixed together. In each experiment several independent 10 gm. portions were taken from the main sample and treated as independent samples, in order to ascertain the limits of variability of the experimental results within a single sample of soil. The total numbers, estimated from the productiveness of the dilution cultures, are given below and are seen to lie well within the limits prescribed by the theoretical factor for the probable error due to random sampling.

*Sample XVII*, 19. vii. 1921:

*Broadbalk, Plot 2.* Three independent 10 gm. portions, A, B and C, taken from main sample:

Estimated numbers of algae.	Sub-sample A	3871
	" B	4082
	" C	3011
	Average	3655

*Sample XIX*, 31. x. 1921:

*Barnfield, Plot 1.* Four independent 10 gm. portions, A, B, C and D, taken from main sample:

Estimated numbers of algae.	Sub-sample A	2359
	" B	1329
	" C	2461
	" D	2100
	Average	2062

## III. THE ALGAE OF BROADBALK WHEAT FIELD.

During the year 1920 an investigation was carried on, by means of the counting method described above, to determine the nature of the soil alga-flora on two of the plots of Broadbalk field, and the various phases in the life histories of the organisms concerned. It was hoped in this way to obtain some information in regard to the extent and distribution of the alga-flora, both on the surface of the ground and in the lower layers of the soil, and to the effects of manurial treatment and of seasonal variation on the flora. Further, it was hoped that the data might yield some evidence in regard to the state in which the algae exist in the soil, whether active or resting.

Broadbalk is a sloping field on which wheat has been grown continuously since 1843. It is divided longitudinally into strips, 7-8 yards wide, which receive separate manurial treatment, the same manure being applied to each strip every year. The plots selected for investigation were:

(a) Plot 2, which ever since 1843 has received an annual dressing of farmyard manure at the rate of 14 tons per acre.

(b) Plots 3 and 4, which have remained unmanured since 1839.

At the top end of each of the two plots a small area was marked off 10 yards in length, and from these small areas samples of soil were taken periodically during the year. The method used for sampling was that devised by Mr H. M. Morris. Four iron plates were driven into the ground at right angles to one another so as to enclose a block of soil 9 in. square. A hole was then dug on one side of the enclosed block and the front plate thus exposed was removed. By means of a flat-bottomed scoop the different layers of soil in the 9-in. block could be removed in succession without risk of mixing them. (For full details and diagrams see Morris, 1922.) For an investigation of the algae, samples of soil were taken by scraping away with a sterilised knife the surface of the soil exposed at different depths, and then removing small portions of the underlying soil from various parts of the exposed area. About 30 gm. of soil was removed for each sample and placed in a sterilised tube to be examined separately. The samples were taken from the unmanured and manured plots alternately until sample VI, after which, for comparative purposes, samples of the top inch layer were always taken from both plots on the same day, the lower layer samples being taken alternately as before.

The first four trials were each made with samples from the top inch,



the sixth inch and the twelfth inch layers of soil, and later experiments with samples from the top, second, fourth and sixth inches. Owing to the use in the first four experiments of a less efficient method of dilution of the soil suspension the experimental error due to random sampling was much higher than in the method finally adopted, and the results of these experiments are therefore less reliable than those of the later ones; they are included, however, in order to give a more complete record.

In the earlier experiments, in addition to the dilution cultures, two series of six cultures were set up containing respectively 5 c.c and 2.5 c.c. of a 1 in 10 suspension of soil, in the hope of obtaining the less frequent forms present in the soil. The results of these earlier experiments showed the relative unimportance of the less frequent species, and cultures of these heavier suspensions were therefore omitted altogether from the later experiments. The mineral salts solution used for making up the soil suspensions was that used in the previous work (Bristol, 1920) diluted to half-strength. Water distilled in a silver still was used for all media, owing to the known toxic action of traces of copper on certain algal species. Aliquot portions of the soil suspensions were transferred by means of sterile pipettes to sterilised vessels containing quantities of specially purified and sterilised sand. The heavier suspensions were cultivated in test-tubes, and in 50 c.c. Erlenmeyer flasks in which the sand was tilted in such a way as to present all gradations between a moist sand and a free-water surface for the growth of the different species. It was found, however, that this method presented no advantages over the test-tube method, which being more convenient was used exclusively in the later experiments.

At the end of a week algae could be observed growing in many of the cultures either with the naked eye or by means of a hand lens, but the cultures were left undisturbed for 3-4 weeks, until the less frequent forms had multiplied sufficiently for identification. The cultures were then examined microscopically and lists made of the species present in each culture.

The identification of the species was at first found to be extremely difficult in the mixed cultures, for a number of the lower algae pass through stages in their life-histories in which they very closely resemble one another; and at the present time there are still a number of forms appearing in the cultures, the recognition of which is uncertain, and which have therefore been neglected in recording the results. In order to facilitate the identification of the species attempts were made to separate them by the use of a solid medium consisting of the mineral

salts solution used for the main body of the work stiffened with 1.2-1.5 per cent. agar<sup>1</sup>. Eleven species were separated from one another in this way, though none of them was obtained in really pure culture, i.e. free from bacteria, and enough information was obtained in regard to their characteristics to facilitate their identification in mixed cultures.

Table I. *Broadbalk algae.*

Species recorded	Plot 2 (Farmyard manure) Depth in inches					Plots 3 and 4 (Unmanured) Depth in inches				
	Top	2nd	4th	6th	12th	Top	2nd	4th	6th	12th
Moss protonema	...	...	...	...	...	x	x	.	.	.
Flagellatae						x	x	x	x	.
<i>Euglena viridis</i> Ehrenb.	...	...	...	...	...	x	x	.	.	.
Chlorophyceae:										
<i>Chlamydomonas muscicola</i>	...	...	...	...	...	x	?	?	?	x
<i>Ch. gloeocystiformis</i> Dill.	...	...	...	...	...	x	?	?	x	x
* <i>Ch. communis</i> Snow.	...	...	...	...	...	.	.	.	.	.
<i>Trochiscia</i> sp.	...	...	...	...	...	x	.	.	x	x
<i>Chlorella</i> spp. incl. <i>C. vulgaris</i> Beij.	...	...	...	...	...	x	x	x	x	x
<i>Scenedesmus</i> sp. near <i>S. costulatus</i> Chod. var. <i>chlorellioides</i>	...	...	...	...	...	x	x	x	.	.
* <i>Dactylococcus bicaudatus</i> A.Br.	...	...	...	...	...	x	.	.	.	.
* <i>Characium</i> sp. (= <i>C. Naegeli</i> A.Br.?)	...	...	...	...	...	.	x	.	.	.
<i>Chlorococcum humicola</i> (Naeg.) Rabenh.	...	...	...	...	...	x	x	x	x	x
<i>Chlorochytrium</i> sp.	...	...	...	...	...	.	.	.	x	.
<i>Pleurococcus vulgaris</i> Menegh. (incl. <i>Cystococcus humicola</i> )	...	...	...	...	...	x	x	x	x	x
<i>Protococcus viridis</i> Ag. (= <i>Pleurococcus Naegeli</i> Chod.)	...	...	...	...	...	x	x	x	x	x
<i>Stichococcus bacillaris</i> Naeg.	...	...	...	...	...	x	x	x	x	x
* <i>Stichococcus</i> sp.	...	...	...	...	...	x	x	x	.	.
* <i>Ulothrix subtilis</i> var. <i>variabilis</i> (Kuetz.) Kirchn.	...	...	...	...	...	.	.	.	.	.
<i>Heterococcus viridis</i> Chod.	...	...	...	...	...	x	x	x	x	x
<i>Bumilleria exilis</i> Klebs	...	...	...	...	...	x	x	x	x	x
<i>Bumilleria sicula</i> Borzi (small form)	...	...	...	...	...	x	.	.	.	.
<i>Tribonema bombycinum</i> (Ag.) Derb. and Sol.	...	...	...	...	...	.	.	.	.	.
Myxophyceae:										
<i>Nostoc muscorum</i> Kuetz.	...	...	...	...	...	x	?	?	x	x
<i>Nostoc humifusum</i> Carmichael	...	...	...	...	...	x	?	?	x	x
<i>Anabaena oscillarioides</i> Born. var. <i>terrestris</i> Bristol	...	...	...	...	...	.	x	.	x	.
* <i>Cylindrocapsa catenatum</i> Ralfs.	...	...	...	...	...	.	.	.	.	.
<i>Cyl. licheniforme</i> (Bory) Kuetz.	...	...	...	...	...	x	.	?	.	.
? * <i>Microchaete tenera</i> Thur. (Young form?)	...	...	...	...	...	.	.	.	x	x
<i>Phormidium tenue</i> (Menegh.) Gomont	...	...	...	...	...	x	.	.	.	.
<i>Ph. autumnale</i> (Ag.) Gomont	...	...	...	...	...	x	x	x	x	.
<i>Ph. laminosum</i> (Ag.) Gomont	...	...	...	...	...	x	x	x	.	?
<i>Lyngbya</i> (a) near <i>L. Lagerheimii</i> (Moebl.) Gomont	...	...	...	...	...	x	.	.	x	.
<i>Lyngbya</i> (b) near <i>L. halophila</i> Hansg.	...	...	...	...	...	x	.	.	x	.
? <i>Symplocococcus muralis</i> Kuetz.	...	...	...	...	...	x	x	x	x	.
Bacillariae:										
* <i>Navicula Balfouriana</i> Grun.	...	...	...	...	...	x	.	.	.	x
* <i>Nav. mutica</i> Kuetz. formae	...	...	...	...	...	x	.	.	.	x
<i>Nav. Atomus</i> Naeg.	...	...	...	...	...	x	x	x	x	x
<i>Hantzschia amphioxys</i> (Ehr.) Grun.	...	...	...	...	...	x	x	x	x	x
* <i>Nitzschia Palea</i> (Kuetz.) W.Sm.	...	...	...	...	...	.	x	x	.	.
<i>Surirella ovalis</i> var. <i>minuta</i>	...	...	...	...	...	x	x	x	x	.
Total number of species	...	...	...	...	...	30	24	21	25	16
						32	18	19	22	13

The species observed in cultures from the different layers of soil are given in Table I, where they are presented side by side for comparison.

<sup>1</sup> Full details of methods suitable for this purpose are given elsewhere (Bristol Roach, 1926 and 1927).

Table I includes all the species that have been identified, even those which have been found only in a single culture of one soil sample. Such species must be regarded as casual inhabitants of no importance in regard to soil fertility, and are marked in the table with an asterisk. The records for the Myxophyceae, as stated above, are possibly incomplete, since the apparent absence of these algae from the second and fourth inch depths may be due to the unsuitability of the culture method employed in dealing with these later samples; their presence at the sixth inch depth was revealed in earlier cultures containing at least eight times as much soil in suspension.

For the more frequent green algae and diatoms, where the records are much more reliable, it is interesting to note that they occur regularly in the lower layers of the soil as well as on the surface, and sometimes in such numbers as to justify the assumption that they must have an important effect on the fertility of the soil. The following table (II) gives a detailed record of the cultures made from a single sample of soil, in order to illustrate the method of setting up the cultures for the purpose of obtaining some information about the frequency of the different species in the soil. The records for all species but two are seen to lie well within the limits imposed for ascertaining the reliability of the results. Of the two species lying outside these limits, the records for *Stichococcus* sp. demonstrate clearly the importance in frequency determinations of setting up an adequately long series of dilutions of a soil suspension which must be made from a sample of soil large enough to be representative of the whole. The presence of this species in culture 35 after a long series of blanks is explicable on the basis of the random sampling of a casual individual, but without the earlier records such an observation might lead to a wholly false estimate of the numbers present. The records for *Protococcus viridis* Ag., on the other hand, are typical of what has often been observed for this species during the experiments. The difficulty of inducing this organism to grow in liquid media is well known, and it is probably this factor rather than random sampling which makes the results so erratic and unreliable.

Further, the absence of blue-green algae from all cultures of the series except Nos. 7-10 is very striking in view of the fact that the surface of this particular sample of soil was covered with a continuous stratum of these organisms almost a millimetre thick. It is evidence of this kind that has necessitated the omission of the blue-green algae from estimates of the numbers of algae present in the different layers of soil.

Table II. *Record sheet for Sample VI a. (Manured Plot, top inch, collected 27. vii. 1920.)*

Degree of dilution of soil suspension; 5 c.c. in each tube	Culture No.	<i>Stichococcus</i> sp.	<i>Bumilleria exilis</i>	<i>Chlamydo-</i> <i>monas</i> sp.	<i>Chlorococcum</i> <i>humicola</i>	<i>Pleurococcus</i> <i>vulgaris</i>	Menegh.	<i>Heterococcus</i> <i>viridis</i>	<i>Chlorella</i> sp.	<i>Protophycus</i> <i>viridis</i> Ag.	<i>Euglena viridis</i>	<i>Hantzschia</i> <i>amphioxys</i>	<i>Surirella ovalis</i> var. <i>minuta</i>	<i>Navicula</i> <i>Atomus</i>	<i>Nostoc</i> sp.	<i>Lyngbya</i> sp.
1/160	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0
	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1/320	10	0	+	+	+	+	+	+	+	+	+	+	+	+	0	+
	11	+	+	+	+	+	+	+	+	+	+	0	+	0	0	0
	12	0	+	+	+	+	+	+	+	+	+	+	+	0	0	0
1/640	13	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0
	14	0	+	+	+	+	+	+	+	+	+	0	0	0	0	0
	15	0	+	+	+	+	+	+	+	0	+	0	0	0	0	0
1/1,280	16	0	+	+	+	+	+	+	+	+	0	0	0	0	0	0
	17	0	+	+	+	+	+	+	+	0	+	0	0	0	0	0
	18	0	+	+	+	+	+	+	+	0	+	0	0	0	0	0
1/2,560	19	+	+	+	+	+	+	+	+	0	+	0	0	0	0	0
	20	0	+	+	+	+	+	+	+	+	+	0	0	0	0	0
	21	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0
1/5,120	22	0	+	+	+	+	+	+	+	0	+	0	0	0	0	0
	23	0	+	+	+	+	+	+	+	+	+	0	0	0	0	0
	24	0	+	+	+	+	+	+	+	0	0	0	0	0	0	0
1/10,240	25	0	+	+	+	+	+	+	+	+	+	0	0	0	0	0
	26	0	+	+	+	+	+	+	+	0	0	0	0	0	0	0
	27	0	+	+	+	+	+	+	+	0	0	+	0	0	0	0
1/20,480	28	0	+	0	0	0	+	+	+	0	0	0	0	0	0	0
	29	0	0	+	0	0	+	+	+	0	0	0	0	0	0	0
	30	0	+	+	+	+	+	+	+	0	+	0	0	0	0	0
1/40,960	31	0	+	0	0	0	+	+	0	0	0	0	0	0	0	0
	32	0	+	0	+	+	+	+	0	+	0	0	0	0	0	0
	33	0	0	+	0	0	+	+	0	0	0	0	0	0	0	0
1/81,920	34	0	+	0	0	+	0	+	0	0	0	0	0	0	0	0
	35	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
	36	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0
1/163,840	37	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
	38	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0
	39	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
1/327,680	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	41	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0
	42	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0
1/655,360	43	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0
Estimated Nos. per gm. of soil		80	8,026	3,316	3,316	3,316	18,138	23,752	407 (?)	617	617	49	49	27	Total 61,893	

Moore and Carter have attempted to estimate the numbers of algal individuals at different depths in the soil by inoculating a series of cultures with progressively smaller samples of soil weighed out separately, and varying in quantity from 5 to 0.01 gm. The method unfortunately is not one in which any great reliance can be placed since it is liable to considerable error on the basis of random sampling, an error which becomes progressively greater as the size of the inoculum diminishes.

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When it is further considered that their estimations are based on observations of a single culture of each weight of inoculum, the probable error of their determinations becomes enormously increased, and their figures must therefore be taken with reserve.

In Table III are given the estimated total numbers per gram of soil of the more important species of green algae and diatoms in a number of different soil samples from Broadbalk field. It was seen from the first four samples that the numbers of algae at the sixth and twelfth inch depths were very much smaller than in the top inch sample, though the numbers were quite considerable at the sixth inch depth in Samples II-IV. At the twelfth inch the clay sub-soil had already been reached and as it is a well-established fact that the biological activity of most

Table III. *Total numbers of algae (Chlorophyceae and Diatoms) per gram in different samples of soil.*

Broadbalk, Plots 3 and 4. Unmanured.						
Sample No.	Date of collection	Top inch	Second inch	Fourth inch	Sixth inch	Twelfth inch
I	6. ii. 20	3,230	—	—	250	66
III	26. iii. 20	36,500	—	—	4,080	330
V	28. vi. 20	15,788	10,422	28,012	4,282	—
VII	29. vii. 20	21,510	—	—	—	—
IX	21. ix. 20	2,797	6,666	8,880	721	—
XI	24. xi. 20	10,447	—	—	—	—
Broadbalk, Plot 2. Manured.						
II	26. ii. 20	30,220	—	—	1,670	166
IV	28. iv. 20	105,550	—	—	2,800	430
VI	28. vii. 20	61,893	27,715	55,747	14,753	—
VIII	21. ix. 20	5,897	—	—	—	—
X	24. xi. 20	91	152	519	53	—
XVI	30. vi. 21	23,694	29,124	16,112	22,458	—

soil organisms is at a maximum at a depth of 3-4 inches, later samples were confined to those layers of the soil where the existence of algae might be more likely to be conspicuous, viz. the top 6 inches. A glance at Table III reveals the fact that the numbers of algae at the fourth inch depth are either significantly greater (Samples IX and X), probably greater (Sample V) or not significantly less (Samples VI and XVI) than the numbers in the top inch sample, and further that the number of organisms in the lower layers of soil varies significantly from sample to sample. Such a result would certainly not be obtained were the algae in the lower layers of the soil mainly present in a resting condition, as has been suggested by Esmarch (1914) and others. In ploughing the field, which is a heavy clay, the plough penetrates to a depth of about 6 in. and turns the vertical block of soil over obliquely. The change of position of the various layers would not bring the fourth inch to the

surface nor replace the fourth inch by the top inch; hence the suggestion that the presence of algae in the lower layers of the soil is due to the burying of surface vegetative forms which thereupon enter into a resting condition is not likely to carry any conviction when the present results are taken into consideration. On the contrary, the results point very clearly to the existence of a definite flora capable of growth and multiplication below the ground. Further evidence on this point is given in Section V of this paper.

Experimental evidence has been obtained that the lateral distribution of the alga-flora over the limited area under investigation did not vary to anything like the same extent as was observed in the samples collected at different times of the year, and there is some indication that the great variations in numbers in the different samples are to some extent expressions of climatic conditions. The high numbers in Samples VI and VII followed a period of heavy rainfall, when the moisture contents of the surface samples were respectively 22.6 and 16.2 per cent. On the other hand, the high numbers of Sample XVI followed a period of severe drought lasting for many weeks and the water content of the soil was only 4.5 per cent. in the different layers. It is perhaps significant, however, that the dominant species in this sample was not recorded from Samples VI and VII. The low numbers in Sample X were preceded by a sharp frost lasting for a fortnight and may possibly be ascribed to that cause. The number of samples examined, however, owing to the cumbersome technique is too small to draw any more serious conclusions in regard to seasonal variation of the numbers of algae.

In Table IV are given the estimated numbers of individuals of the more frequently occurring species in a number of different samples. From this table it is seen that although the results are less consistent than when total numbers are considered, there is often a significantly larger number of individuals in the lower layers than on the surface, or no significant diminution. This is specially noticeable in the unmanured plot for the following species: *Chlorococcum humicola*, *Pleurococcus vulgaris* Menegh., *Chlorella* sp., *Bumilleria exilis*, *Heterococcus viridis*, *Protococcus viridis* Ag. and *Chlamydomonas muscicola*. In the other species the differences are either not conspicuous or are significantly in favour of the top layer. One is tempted to suggest, therefore, in comparing the results for the different species, that the alga-flora of the soil is made up of two types of organisms, the surface forms and the true soil forms which appear to be equally at home on the surface and in the lower layers.

Table IV. *Estimated numbers of commonest species in different samples of soil.*

Name of species	Broadbalk, Plots 3 and 4 (unmanured)						Broadbalk, Plot 2 (farmyard manure)					
	Sample No.	Date of collection	Top inch	Second inch	Fourth inch	Sixth inch	Sample No.	Date of collection	Top inch	Second inch	Fourth inch	Sixth inch
<i>Chlorococcum humicola</i> (Naeg.) Rabenh.	III	26. iii. 20	20,000	—	—	1,100	IV	28. iv. 20	66,000	—	—	1,680
	V	28. vi. 20	2,072	1,300	2,072	407	VI	28. vii. 20	3,316	5,325	18,138	5,325
	VII	29. vii. 20	514	—	—	—	VIII	2. ix. 20	257	—	—	—
	IX	21. ix. 20	181	1,300	1,030	37	X	24. xi. 20	0	13	81	26
	XI	24. xi. 20	6,780	—	—	—						
<i>Pleurococcus vulgaris</i> Menegh.	III	26. iii. 20	1,300	—	—	830	IV	28. iv. 20	3,300	—	—	110
	V	28. vi. 20	141	648	162	80	VI	28. vii. 20	3,316	514	648	181
incl. <i>Cystococcus humicola</i> Naeg.	VII	29. vii. 20	49	—	—	—	VIII	2. ix. 20	11	—	—	—
	IX	21. ix. 20	11	407	37	11	X	24. xi. 20	—	—	—	—
	XI	24. xi. 20	2	—	—	—						
<i>Chlorella</i> sp.	V	28. vi. 20	11,000	5,325	4,196	1,030	VI	28. vii. 20	23,752	14,171	23,752	1,642
	VII	29. vii. 20	18,138	—	—	—	VIII	21. ix. 20	4,196	—	—	—
	IX	21. ix. 20	1,642	514	3,316	162	X	24. xi. 20	12	26	325	6
	XI	24. xi. 20	205	—	—	—						
<i>Bumilleria exilis</i> Klebs	III	26. iii. 20	3,500	—	—	500	IV	28. iv. 20	1,000	—	—	200
	V	28. vi. 20	204	37	407	49	VI	28. vii. 20	8,626	648	648	257
	VII	29. vii. 20	817	—	—	—	VIII	21. ix. 20	648	—	—	—
	IX	21. ix. 20	257	648	1,030	11	X	24. xi. 20	6	20	51	4
	XI	24. xi. 20	1,642	—	—	—						
<i>Heterococcus viridis</i> Chod.	III	26. iii. 20	1,500	—	—	550	IV	28. iv. 20	1,000	—	—	330
	V	28. vi. 20	1,030	817	5,325	1,642	VI	28. vii. 20	18,138	5,325	4,196	5,325
	VII	29. vii. 20	1,642	—	—	—	VIII	21. ix. 20	162	—	—	—
	IX	21. ix. 20	323	3,316	2,619	407	X	24. xi. 20	49	20	7	6
	XI	24. xi. 20	414	—	—	—						
<i>Protococcus viridis</i> Ag. (= <i>Pleurococcus Naegelii</i> Chod.)	III	26. iii. 20	10,000	—	—	1,100	IV	28. iv. 20	1,000	—	—	500
	V	28. vi. 20	323?	1,642	14,171	648	VI	28. vii. 20	407	514	6,780	1,030
	VII	29. vii. 20	101	—	—	—	VIII	21. ix. 20	0	—	—	—
	IX	21. ix. 20	27	49	27	11	X	24. xi. 20	0	—	—	—
	XI	24. xi. 20	64	—	—	—						
<i>Chlamydomonas muscicola</i>	V	28. vi. 20	648	648	2,072	407	VI	28. vii. 20	3,316	323	648	101
	VII	29. vii. 20	181	—	—	—	VIII	21. ix. 20	323	—	—	—
	IX	21. ix. 20	181	323	648	63	X	24. xi. 20	6	10	1	—
	XI	24. xi. 20	204	—	—	—						
<i>Stichococcus bacillaris</i> Naeg.	V	28. vi. 20	181	5	37	—	VI	28. vii. 20	80	162	37	—
	VII	29. vii. 20	19	—	—	—	VIII	21. ix. 20	257	—	—	—
	IX	21. ix. 20	101	80	162	19	X	24. xi. 20	10	23	12	—
	XI	24. xi. 20	63	—	—	—						
<i>Hantzschia amphioxys</i> (Ehr.) Grun.	V	28. vi. 20	37	—	—	—	VI	28. vii. 20	49	80	27	19
	VII	29. vii. 20	27	—	—	—	VIII	21. ix. 20	27	—	—	—
	IX	21. ix. 20	37	5	11	—	X	24. xi. 20	8	23	20	3
	XI	24. xi. 20	51	—	—	—						
<i>Navicula Atomus</i> Naeg.	V	28. vi. 20	37	—	—	19	VI	28. vii. 20	27	49	37	37
	VII	29. vii. 20	—	—	—	—	VIII	21. ix. 20	11	—	—	—
	IX	21. ix. 20	—	5	—	—	X	24. xi. 20	—	6	8	4
	XI	24. xi. 20	13	—	—	—						

The numbers of green algae recorded from these experiments are of an order quite comparable with those of the protozoa on the same plots, and when it is considered that the blue-green forms may be just as numerous at least in the surface layer, as a visual inspection would lead one to suspect, the influence of algae on soil fertility cannot be disregarded. In view of the wide distribution in this country of certain species of soil diatoms (Bristol, 1920), it is surprising that the numbers of individuals present is low in most of the samples so far examined.

In all of the samples taken, with the exception of those of Nov. 24, the numbers present in the unmanured plot fell considerably below those in the corresponding samples from the manured plot. On Nov. 24, following a fortnight of sharp frosts, there was an unexpected rise in numbers on the unmanured plot, due chiefly to the multiplication of the single species *Chlorococcum humicola*, while the algae on the manured plot remained at a minimum. No explanation of this difference can be offered at present as the evidence is too scanty, nor can it be stated whether the usual superiority in numbers of the manured plot is a direct manurial effect, or whether it may not be partly an indirect effect due to the fact that the manured plot has a high content of organic matter and has consequently a higher water content than the unmanured plot. That manurial treatment has a direct effect upon the alga-flora was patent to the naked eye during the month of July when there was a continuous heavy rainfall. By the last week in the month there was a conspicuous growth of algae on the surface of the soil over the whole field: on the manured plot there was a greenish black gelatinous layer nearly a millimetre thick extending almost continuously over the surface of the ground, whereas on the unmanured plot the algae formed merely a very thin and much paler green stratum scarcely visible to the naked eye. Between these two extremes all gradations could be observed on the other plots, those with the highest nitrogen content giving rise to the greatest growth of algae.

Two very interesting species are the yellow-green algae *Bumilleria exilis* Klebs and *Heterococcus viridis* Chod., in both of which there seems to be a marked difference of behaviour in the manured and unmanured plots; for both species are at a maximum at the fourth inch depth in the unmanured plot, while in the manured plot conditions for their growth appear to be much more favourable on the surface. In the present limited state of our knowledge one is at a loss to suggest a possible explanation of this anomaly, but it is perhaps significant that these species, of which the growth is greatly stimulated in pure culture by the addition of certain carbohydrates, have been shown by Chodat (1913) to be adversely affected by the presence in the medium of small quantities of soluble peptone, whereas less available protein in the form of gelatine has no deleterious effect. One is tempted therefore to suggest that the abnormally large amount of organic matter in Plot 2 may have rendered the soil less suitable as a medium for their vigorous growth than that of the unmanured plot.



## IV. THE ALGAE OF BARNFIELD.

Mangolds have been grown in Barnfield annually for the last fifty years. Samples of soil from the lower end of the farmyard manure plot have been taken on a number of occasions, and have consisted of a mixture of several 1-6 inch borings obtained with a screw-sampler or a hollow cylindrical tube borer. The separate cores of soil were mixed in a sterile bottle, and after transference to the laboratory were passed through a 3 mm. sieve and thoroughly mixed on a large sheet of clean paper. Representative 10 gm. samples were then taken from the whole and examined culturally in the usual way.

The alga-flora of this field appears, from the limited number of samples examined, to differ from that of Broadbalk, situated nearly a mile away, chiefly in a conspicuous shortage of blue-green species; the important constituent species of green algae and diatoms do not differ from those of Broadbalk, though they are fewer in number. Owing to the very different crops habitually grown on the two fields (wheat and mangolds) the cultural operations to which they are subjected are quite different: the operations on Barnfield aim at reducing the surface of the soil to a fine mulch which is probably unsuitable for the growth of blue-green algae.

The following species have been identified:

Moss protonema

Flagellatae.

*Euglena viridis* Ehrenb.

Chlorophyceae.

*Chlamydomonas gloeocystiformis* Dill.

*Chlorella* sp.

*Chlorococcum humicola* (Naeg.) Rabenh.

*Pleurococcus vulgaris* Menegh.

*Protococcus viridis* Ag.

*Ulothrix* sp.

*Vaucheria* sp.?

*Heterococcus viridis* Chod.

*Bumilleria exilis* Klebs

? *Bumilleria sicula* Borzi

Myxophyceae.

*Anabaena* sp.

? *Phormidium Bohneri* Schmidle

*Phormidium autumnale* (Ag.) Gomont

*Lyngbya Kuetzingii* Schmidle

Bacillariaceae.

*Navicula mutica* formae?

*Navicula Atomus* Naeg.

*Navicula exilissima* Grun.

*Hantzschia amphioxys* (Ehr.) Grun.

*Nitzschia Palea* (Kuetz.) W.Sm.

*Surirella ovalis* var. *minuta* and another larger form?

A series of fifteen samples, each consisting of a mixture of four fairly widely separated 1-6 inch borings, and taken at 4, 6, 8 and 10 a.m. and 12 noon respectively on three successive days in July 1921, within a special plot 8 ft. square at the lower end of the manured plot on Barnfield, yielded the same species in every series of cultures, showing that over a limited area the alga-flora is uniform in distribution. The experiment was preceded by a severe drought lasting for many weeks and the soil was in a very dry and friable condition. The total numbers of algae per gram of soil in the various samples are given below, and are seen to be rather low:

	First day	Second day	Third day
4 a.m.	1043	676	3368
6 "	5138	712	2700
8 "	3350	2037	2301
10 "	1602	801	3725
12 noon	1574	875	3152
Average	2541	1020	3049

The average for the second day is significantly lower than those for the other two days, suggesting that even under these extremely dry conditions there may possibly be some fluctuation in the numbers of individuals from day to day, but the changes are not striking and the data are too scanty for emphasis.

Other samples taken in October yielded a total of about 2000 green algae and diatoms per gram of soil and in November a total of only 850. A sample taken in June 1926 on the other hand, after a few weeks of dry weather, when the top inch of soil was dry and friable and there was no visible sign of algae growing on the surface, yielded a total of green algae and diatoms not less than 127,830 per gram of soil chiefly made up of the following species: *Chlorella* sp. 75,000, *Chlorococcum humicola* 14,170, *Heterococcus viridis* 11,000, *Pleurococcus vulgaris* Menegh. 3320, *Bumilleria exilis* 2620, *Chlamydomonas gloeocystiformis* 60?, *Navicula exilissima* (?) 18,140, *Hantzschia amphioxys* 2070, *Navicula mutica* (?) 320, *Surirella ovalis* var. 260, *Nitzschia Palea* 50, *Phormidium Bohneri* Schmidle 820.

The total number of diatoms in this sample (nearly 21,000) is conspicuously higher than in any other sample examined by this method. The high figures obtained for most species on this occasion suggest that the uniformly low figures obtained from the earlier samples are to be ascribed to the unfavourable weather conditions prevailing previously to the taking of the samples and not to any inherent poverty in the flora itself.

V. ARE THE ALGAE OF THE SOIL PRESENT IN A RESTING  
OR A VEGETATIVE STATE?

The results cited in the earlier sections of this paper point clearly to the existence of a definite flora not merely quiescent but capable of growth and multiplication below the surface of the ground, though a proof of this fact is essential to the subject. Circumstantial evidence of quite a different character points to the same conclusion. In the experiments carried out in 1915-19 soils were used which had first been carefully air-dried for at least a month, in order to convert all vegetative forms into resting cysts and to cut out non-resistant species. Cultures of such soils never gave rise to algae until 6-10 weeks had elapsed after the cultures had been set up. In cultures of fresh soils, on the other hand, algae can often be seen with the naked eye or with a hand lens at the end of a week, a difference which is evidently due to the presence of vegetative forms in the fresh soils. Further support of this suggestion was obtained from a series of four exactly parallel cultures of the first twelfth inch sample of the manured plot, in which the algal numbers were very low. Of the four cultures, three produced a visible stratum of algae within three weeks of being set up, the relative slowness of its appearance being probably due to the small number of cells present in each culture; in the fourth culture, on the other hand, there was no visible growth until the twelfth week, when three of the same species of algae appeared as were already growing in the other cultures, apparently arising from latent spores present in the inoculum.

On the basis of these observations, a method has recently been devised by means of which a rough idea may be obtained of the proportion of vegetative to resting cells in a given sample of soil. The method depends on the rapid desiccation of the soil by means of a stream of dried air, the tube containing the soil being kept in a water bath at 35° C. By these means it is intended to impose conditions only comparable with a dry wind on a hot day, such as would be fatal to actively growing thin-walled vegetative cells but quite harmless to resistant resting cells, and to carry out the whole process so quickly as to render negligible errors due to naturally occurring changes. Details of one experiment are given below.

A 1-6 inch sample of soil, sufficient to fill a 16 oz. bottle, was taken from a cottage garden in Harpenden, and after being passed through a 3 mm. sieve was thoroughly mixed on a sheet of sterile paper. Two equal quantities of soil (6 gm.) were then weighed out and examined

as separate samples by the quantitative method described in Section II. An extended series of dilution cultures of the first sample, A, was set up immediately; while the second sample, B, was placed in a small sterile U-tube which was then attached to the apparatus illustrated in Fig. 1 and submerged in a water bath at 35° C., when a stream of dry sterile air at laboratory temperature was drawn through it by means of a filter pump.

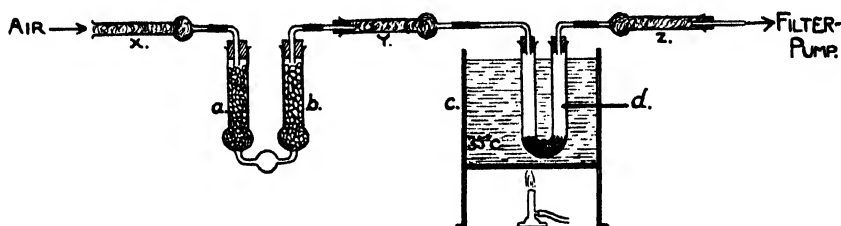


Fig. 1. Apparatus for rapid drying of soil sample. *a, b*, bulb U-tube containing granular  $\text{CaCl}_2$ ; *c*, water-bath at 35° C.; *d*, U-tube containing soil sample; *x, y* and *z*, calcium chloride tubes filled with cotton wool plugs to prevent entrance of organisms during drying; *x* and *a, b*, unsterilised; *y, d, z* and their connecting tubes previously sterilised.

The water in the sample of soil rapidly evaporated and condensed again in the sterile plug *z*, from which it again evaporated in about 2 hours. The calcium chloride in the side *a* of the bulb tube became moist on the surface of the granules, but that on the side *b* remained quite dry, showing that the stream of air had been completely dried. The passage of air was continued for about 5 hours, when the sample of soil appeared to be thoroughly dry and the particles crumbled to pieces immediately on the addition of the culture solution. The mixture was, however, shaken for the usual half hour and a series of dilution cultures was prepared in exactly the same way as for the untreated sample.

The records made of the species present in the cultures of both series are included in Table V, where they are placed side by side for comparison. It is seen that the effect of desiccation is completely to kill off all the diatoms, for though occasional empty frustules were observed in the soil sediment in the cultures of sample B, live individuals of a single species, very few in number, were found in only one culture. The blue-green algae, so far as one can judge, were all unaffected except the little species identified as *Oscillatoria* sp.; this had extremely delicate filaments around which there seemed to be no trace of a mucous sheath, hence the extreme effect of desiccation is not surprising; all of the other species observed possessed stout sheaths that would make them highly

[illegible]



resistant. Among the green algae the species least affected by drying is *Pleurococcus vulgaris* Menegh. which is cut down to 1/2-3 of its numbers in the fresh sample, a difference just large enough to be outside the limits of variation probable as a result of random sampling. *Chlorococcum humicola* was reduced to one-twelfth of its numbers, while in *Chlorella* sp. and *Heterococcus viridis* more than 99 per cent. of cells were killed off. In *Bumilleria exilis* only a few cells survived, while the other less frequent species were completely killed off in the dried sample.

In view of the comparatively uniform results obtained from separate representative portions of a single well mixed sample (see p. 566) and of the thoroughness of the initial mixing of the soil sample in the experiment under consideration these results cannot be explained on any other basis than the killing off of the vegetative forms by the rapid drying of the soil, while the more resistant resting cells were unaffected. The experiment may therefore be regarded as a proof of the existence of the algae within the soil in a vegetative state.

#### VI. BIOLOGICAL NOTES ON SOME OF THE MORE INTERESTING SPECIES.

*Moss protonema* was observed comparatively rarely in the dilution cultures, but was obtained from all three localities examined. Its apparent infrequency is possibly attributable to the unsuitability of the dilution method for adequately breaking up the filaments.

*Chlamydomonas gloeocystiformis* Dill was obtained from many of the samples from both Broadbalk and Barnfield, but was never present in great numbers. It was quite typical and all stages of its life-history as described by Dill (1895) and by Chodat (1902) were observed in the cultures, but it was most frequently present in the *Gloeocystis* condition; the motile *Chlamydomonas* state was only occasionally observed. Fritsch and Salisbury (1915) have recorded *Gloeocystis vesiculosus* Naeg. as a dominant constituent of the surface flora of burnt patches on Hindhead Common. This observation taken in conjunction with the fact that *Ch. gloeocystiformis* was generally present in my cultures in the *Gloeocystis* state seems to lend support to West's suggestion (1916, p. 172) that *Gloeocystis vesiculosus* Naeg. is probably the palmelloid state of a species of *Chlamydomonas*.

*Trochiscia* sp. This alga has been recorded as a separate species, but it is very doubtful, in my opinion, whether it should be regarded as such. For the alga was observed only in the older cultures and then

only in small numbers, and it is possible that the cells so recorded were resting cells of one of the other species. Chodat (1902, Fig. 196), for example, figures a *Trochiscia*-state for the species *Protococcus viridis* Ag., very similar to the cells in my cultures; and since this latter species was generally present in the cultures the two may be merely different stages of the same alga.

*Chlorella* spp. The algae so recorded included at least two forms, *Chlorella vulgaris* Beij. and another form which exhibited in its adult stage a fragmentation of the chloroplast. In the younger stages it was impossible to distinguish between the two forms and they were therefore included together in making the records. In cultures grown in diffuse light, the young stages of these species could not be distinguished easily from the young stages of *Heterococcus viridis*, but it was found that after some hours' exposure of the cultures to bright light, the addition of dilute iodine solution to the mounted material served to distinguish the two types of cells, by demonstrating the presence of a minute pyrenoid in those of *Chlorella*. These species grew in considerable quantities in cultures of many of the more dilute suspensions, but they were found in the cultures of the higher suspensions only after prolonged search, the effect of competition being very conspicuous. Desiccation of the soil has also a very deleterious effect on these species. Unidentified drawings of chlorelloid cells were made at the first examination of the cultures from four only of the soil samples described in my earlier papers, and it is probable that the combined effects of desiccation and competition served to exclude these algae from the rest of these cultures.

*Characium* sp. It is not likely that this alga can be regarded as a true soil organism for it was observed only in a single culture of one soil sample. It approached most nearly to *C. Naegelii* A.Br., but differed in being rather smaller, in showing a tendency towards a unilateral distortion of some of the larger cells, and in its stipe which was usually only  $2\mu$  long and was sometimes slightly expanded at the base to form a minute disc of attachment.

*Chlorococcum humicola* (Naeg.) Rabenh. is one of the most frequent of the soil algae, as many as 55,000 individuals per gram having been obtained from a single sample of cottage garden soil. Owing to its mode of multiplication by zoogonidia or by thin-walled aplanospores and to its tendency to enter into a palmelloid state, the alga is very sensitive to desiccation, and it is therefore not surprising to find that after a prolonged drought the numbers were as few as 25 individuals per gram



of soil (Broadbalk, Sample XVI, top inch). In 1919 I gave a full account of a Malay form of this alga (Bristol, 1919) from a culture which appeared to contain this species alone. A wider experience has convinced me that there were possibly two very similar species present, the second one being probably *Cystococcus humicola* Naeg. Unfortunately the original material has been lost and it is therefore impossible to verify this suggestion, but the diagrams which lead me to suspect the error are Nos. 23 *d* and 24, in which the nucleus is very different in size and structure from that of the rest of the cells.

Moore and Carter have recently criticised my account on two other points, but neither criticism in my opinion is sound. They say, without giving any reason: "There is some doubt as to whether the large cells mentioned by Bristol really belong to this species. They possibly belong to *Chlorochytrium*." Now a study of the cytological characters of several species of the genus *Chlorochytrium* (Bristol, 1917, 1920 *b*) has shown that their cells contain a single large nucleus situated somewhere near the centre of the cell, whereas the larger cells under discussion were all of the multinucleate type illustrated in Figs. 18 and 19 of my 1919 paper. No possible confusion could have arisen in the stained preparations between cells of such totally different types, even if such an error were possible in the living material.

Secondly, Moore and Carter would identify my Figs. 27 and 28 with their species *B*, and call it the palmelloid state of a species of *Chlamydomonas*. I am quite unable to understand this suggestion, for at no time during the four years that this alga was under observation was there observed in the cultures a single cell of the *Chlamydomonas* type. Moreover, the changes through the successive stages illustrated in Figs. 25–28 were actually observed under the microscope in hanging drop cultures containing initially a single group of cells such as that drawn in Fig. 25; and the fusion of pairs of these motile gonidia, with the gradual formation of typical *Chlorococcum* vegetative cells as illustrated in Fig. 31, was also watched under the microscope. During the last five years I have had in pure culture a small species of *Chlorococcum*, isolated from a Swiss soil, which when inoculated into a mineral salts solution rapidly forms zoogonidia. After some time the zoogonidia in the culture come to rest on the sides of the vessel and in a few weeks a gelatinous palmelloid stratum is produced, the cells of which in older cultures gradually assume the adult *Chlorococcum* form. On mineral salts agar this palmelloid state is the dominant form of the alga; when transferred to distilled water on a slide the palmelloid cells acquire cilia

and rapidly swim out of the gelatinous matrix. There appears to be no reasonable basis for Moore and Carter's emendation of my description.

*Chlorochytrium* sp. The life-history of this organism was not followed sufficiently closely to identify it with certainty with any described species. The cells observed were rather small, 25–50 $\mu$  in diameter, and sub-spherical with a single broad pectosic projection on the outside of the wall; they contained a large central pyrenoid and radiating chloroplast similar to that described (Bristol, 1920 *b*) for *C. paradoxum* (Klebs) G. S. West. It differed, however, from the normal form of this species in that the only mode of multiplication observed was by numerous small aplanospores instead of by zoogonidia, but granules of red pigment were formed in the centre of the cell as in the typical form.

*Pleurococcus vulgaris* Menegh. occurred in all the samples examined. It was observed to assume all the different forms described and figured by Chodat (1902, Figs. 191–194, and 1909, Pl. II) at different stages of its life-history. It is possible that my records of this species include also those of the independent species, *Cystococcus humicola* Naeg., but in the mixed cultures it was impossible to distinguish between the unicellular forms of the two algae, and no attempt was therefore made to identify them separately. It was observed that the aplanospores did not always develop directly into normal vegetative cells but often became enveloped in mucilage and entered into a palmelloid state, similar to that described by Moore and Carter for their Species C to which they possibly attach certain "*Protococcus*-like" stages. Chodat does not mention such a palmelloid condition for *Pleurococcus vulgaris*, and it is possibly a stage in the life-history of the true *Cystococcus humicola*. The cells of this species could always be distinguished from those of *Protococcus viridis* Ag. with which they were often mixed by the addition of iodine, which demonstrated clearly the presence of a central pyrenoid and a stellate chloroplast.

*Protococcus viridis* Ag. = *Pleurococcus Naegelii* Chod. (1902, 1909 and 1913). This alga was obtained from all of the Broadbalk and Barnfield samples, but was absent from the cottage garden. It was usually present in the regularly divided packets of cells typical of the species, but a filamentous arrangement of the cells was not infrequent, and in a number of cultures delicate branched filaments were formed exactly similar to those figured by Chodat (1902, Fig. 197).

*Heterococcus viridis* Chod. has been obtained from all of the samples examined and was often present in considerable numbers. It is extremely sensitive to competition in cultures and in a few weeks may be com-

pletely killed off by more vigorously growing species, even though it may have been fairly plentiful in the early stages. It has been obtained in unialgal culture, and all stages of its life-history as described by Chodat (1919, 1913) have been observed both on agar plates and in the soil cultures. The species was usually present in the *Botrydiopsis* stage, but filamentous forms have been obtained both on mineral salts agar and on the surface of ordinary aqueous soil cultures. Zoogonidia with two unequal cilia have been observed on only two occasions, once in a soil culture and once on an agar plate, the usual method of multiplication being by means of aplanospores which may be of very unequal sizes, even within the same mother-cell.

This species was not recorded in my earlier lists although the drawings made at that time show that it was probably present in the early stages of the cultures of eleven of the soils examined, but it became completely overgrown in the later stages, and the incomplete observations were insufficient to determine its identity with certainty. The alga may be distinguished in a mixed culture from most of the other organisms which it closely resembles by exposing the algae to sunlight for a time and then adding dilute iodine solution, when its cells merely acquire a brownish coloration, in contrast to the starch-containing cells of the other species (*Chlorella*, *Cystococcus*, *Protococcus*, *Dictyococcus*, etc.).

*Bumilleria sicula* Borzi. It is possible that this species may have been present in the cultures more frequently than appears from the records. It was rather smaller than the typical form, but otherwise agreed entirely with the descriptions. Its identification therefore in the filamentous state was quite easy; but the filaments showed a marked tendency, both in the aqueous soil cultures and in unialgal cultures on agar plates, to become disarticulated into single cells which often assumed very abnormal shapes, making their identification extremely difficult in mixed cultures. At other times on agar plates the cells of whole filaments were observed to divide up simultaneously into spherical aplanospores, 2-8 within each cell according to its length; these when liberated by the fragmentation of the cell-walls into H-shaped pieces were almost indistinguishable from the corresponding stage of *B. exilis* Klebs or from isolated cells of *Heterococcus*. It is possible therefore that the records are incomplete in regard to this species.

## VII. SUMMARY.

An account is given of an investigation of the alga-flora of four English soils by means of dilution cultures of freshly gathered samples of soil from the top, second, fourth, sixth and twelfth inch depths and from the top 6 in. mixed. A counting method is described applicable to the green algae and diatoms, by means of which it is shown that these algae are distributed throughout the top 12 in. of soil, though at the sixth and twelfth inch depths they are considerably less numerous than nearer the surface. At the fourth inch depth the numbers of individuals are not significantly smaller than on the surface and may be even greater.

The unmanured plot of Broadbalk wheat field was found to contain the same main species as the adjacent farmyard manure plot but was poorer in numbers of individuals. Thirty-five species are described from each plot; they seem to be divisible into two groups, the true soil forms and casual species. Of the true soil forms some grow equally well on the surface and in the lower layers, whereas others are more numerous on the surface than within the soil. The same main types were also obtained from Barnfield and from a cottage garden, but the blue-green species were less conspicuous in both of these soils.

Experimental evidence is given to show that many of the algae of the soil exist in a vegetative condition rather than a resting condition. Biological notes are made on some of the more important or interesting soil species.

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